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PROCEEDINGS OF THE

1964

COTTONSEED PROCESSING CLINIC

Held at New Orleans, Louisiana
February 17 and 18, 1964

Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

FOREWORD

The USDA's Agricultural Research Service wants to assure that the nation will receive maximum benefit from its research programs. Utilization research is constantly developing new ideas and products which show great possibilities of benefiting growers, industry, and consumers.

The Cottonseed Processing Clinic is sponsored annually by the Southern Utilization Research and Development Division in cooperation with the Mississippi Valley Oilseed Processors Association, Inc. Its purpose is to acquaint representatives of the cottonseed industry with current developments in utilization research and to provide for an exchange of information that will benefit future research.

These proceedings report the statements presented by the various speakers during the 1964 Clinic and give an account of the discussions during the three panels.

C. H. Fisher
Director, Southern Utilization Research and Development Division

GENERAL CHAIRMAN
Bruno H. Wojcik

CO-CHAIRMAN
Myron D. Kolb

CONFERENCE COORDINATOR
Beatrice A. Sharar

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WELCOME

by
C. H. Fisher
Southern Utilization Research and Development Division

Mr. Chairman and members of the Conference:

It is again my honor and pleasure to welcome a splendid audience at the opening of a Cottonseed Processing Clinic. We in the Southern Division look forward to these annual Conferences. We are proud and grateful because we have the privilege of cosponsoring these important meetings and participating in them.

It is fitting and fortunate that the cottonseed industry is interested in research and progress. We are in an exciting era marked by many changes. Catalyzing these changes is a research and development program in the U. S. that will cost about $20 billion in 1964. This is more than 3 percent of our Gross National Product.

The distribution of this vast research and development effort among industries and commodities is not uniform. A large proportion of the total R&D funds is invested in glamour fields such as those represented by synthetic fibers, plastics, and space activities.

Nevertheless, substantial progress has been made with both cottonseed and cotton. We now possess much more basic information about cottonseed and cotton lint. New and better products are being developed, and industry problems are being solved.

In my opinion, industry deserves much credit for this progress. Not only is the industry conducting research of high quality, but it also is working together--and cooperating with other groups--to achieve greater efficiency.

Cooperative utilization research on cotton has created or maintained large markets for cotton. It is estimated that this utilization research--by many organizations--is responsible for the sale of more than 2 million bales of cotton annually or about one-fourth of the total domestic market.

This achievement with cotton lint has, of course, had an impact on the cottonseed industry. It has had an impact also on the starch industry, which supplies starch for the sizing of cotton textile products. That is, the maintenance of markets for cotton has also maintained a large market for starch, perhaps as much as 100 million pounds per year.

I now wish to call your attention to some recent and important developments in the Department of Agriculture.

As many of you know, Dr. Leo A. Goldblatt was appointed during April of the past year to the important position of Chief of the Oilseed Crops Laboratory. This appointment has greatly strengthened our program. Dr. Goldblatt was a member of the Southern Division before going to the Western Division in January 1959. We are delighted, and we are fortunate, because he returned.

Dr. Bruno H. Wojcik, formerly with Hooker Chemical Co. and Olin Mathieson Co., joined our staff last October as Assistant Director for Industrial Development. He is already proving to be a valuable member of our organization, and we are greatly pleased because he was willing to make the transition from industry to the Department of Agriculture.

Perhaps all of you know that the Congress appropriated more money this year for utilization research. A substantial part of the increase is being used to strengthen cotton and cottonseed research.

Funds were provided also for the construction of a fifth regional laboratory. This laboratory will be located at Athens, Georgia. Additional facilities will be constructed also at the existing four regional Laboratories. Thus additional space and facilities are being provided for utilization research. Although we cannot predict the future, one could infer that utilization research might undergo a considerable expansion over the next several years.

Information about the new Southeastern Laboratory to be constructed at Athens, Ga., is in Senate Document 34 and in a press release of February 6, 1964. According to the press release:

> "This new laboratory will provide modern research and pilot-plant facilities for a concerted effort to find new improved uses of poultry and eggs, fruits and vegetables, and other farm commodities of the Southeastern region. Cost of the facility, including basic laboratory furnishings, is estimated at $9-1/2 million."

Construction will be financed with funds appropriated to USDA last December. This will be the first major regional laboratory devoted to utilization research and development authorized to be constructed since 1938.

Before concluding, I wish to thank everyone contributing to this Conference. This includes our visitors for meeting with us, having an interest in our work, and providing help on many occasions. I wish to express appreciation also to the officers of the Mississippi Valley Oilseed Processors Association and to Dr. B. H. Wojcik and his staff for planning and arranging the Cottonseed Processing Clinic.

OPENING REMARKS

by
Myron D. Kolb
President
Mississippi Valley Oilseed Processors Association, Inc.

Thank you. --- It isn't everywhere that we oil mill people can go and be assured of as warm a welcome as Dr. Fisher has extended to us. We want Dr. Fisher and all of his staff to know that we appreciate his welcoming words.

Much has been said and written of the increased pace of the modern world. Most of us here today are familiar with the feeling of time passing faster as we grow older but the speeded-up pace of technology in the world today is something in addition to that feeling, and is a fact which all of us here are called on to cope with in our day-to-day operations and also in the plans which we must make for the future. To do this properly we need more and more information and we frequently find that our past experience does not provide us with the facts that we must have. When that happens we must search elsewhere for the information that we need. The Southern Utilization Research and Development Division is one of our important sources of information, both on current problems and future trends. In fact, through their work they are creating some of these future trends right now. We want you laboratory people to know that we recognize and appreciate the value to us of the work that you have done and are doing.

I am sure that everyone has already checked his printed program several times to see what is in store for us at this year's Cottonseed Processing Clinic, and is ready to get on into the meat of the program. We realize that Dr. Fisher and his staff have put a lot of thought and hard work into the subject matter of our 1964 Clinic, and we want them to know that we deeply appreciate their efforts in our behalf. For all of us, Dr. Fisher, I say "Thank you".

CURRENT COTTONSEED RESEARCH AT THE SOUTHERN DIVISION IN THE OILSEED CROPS LABORATORY

by
Leo A. Goldblatt
Southern Utilization Research and Development Division

I would like first to join the others who have preceded me in welcoming you. This is my first meeting with your group. I gather this is your thirteenth, which makes it a good place for me to come in. It is a pleasure to be with you and I appreciate the privilege of taking part in your discussions.

My assigned topic is current cottonseed research at the Southern Division. I interpret that to mean a description of the research that is now going on and current plans for future research rather than how the research is being conducted or the specific details of results that have recently been obtained, although you will get some of those too.

Research on cottonseed is conducted in at least three different laboratories in the Southern Division. Research on cottonseed is being conducted in my own laboratory, the Oilseed Crops Laboratory; in Mr. Vix's Engineering and Development Laboratory; and in Dr. Altschul's Seed Pioneering Laboratory. The reason I was somewhat indefinite in saying at least three laboratories is that we sometimes cooperate with other Laboratories who help us in our research. For example, I know that our Oilseed Crops Laboratory and also Dr. Altschul's Laboratory call on Mr. O'Connor's Cotton Physical Properties Laboratory for cooperative work. Although research on cottonseed may not be initiated in Mr. O'Connor's Cotton Laboratory nevertheless cooperative research on cottonseed is being conducted there. That is why I used the rather indefinite expression--at least three laboratories.

All I plan to discuss is current research in the Oilseed Crops Laboratory. I know your primary interest is in cottonseed. That is the area which takes most of our research effort but it might be worthwhile to take just a couple of minutes to say a few words about the rest of our program. Our work is concerned primarily with oilseeds produced in the Southern Region. That means cottonseed, peanuts, and tung. We are also conducting research on what are known as New Crops Oilseeds. These are oilseeds that are not produced commercially at the present time but which appear to have potential agronomically and for utilization, especially industrial utilization. The basic thought here is that we have an adequate supply of edible oils, but growing oilseeds to produce industrial oils may supply farmers with entirely new markets. Farmers could then use acres now used to produce crops that are in surplus to produce industrial raw materials. In general, the oilseeds in the new crops program produce oils that are very unusual in some respect and that have industrial potential. For example, one of our new oilseed crops is cuphea, that produces an oil very rich in capric acid and is a 10-carbon atom acid closely related to lauric acid. We import hundreds of millions of pounds of lauric containing oils like coconut oil every year, primarily for use in industry.

Now to return to cottonseed. What are we doing with cottonseed? Let us consider the oil and the meal separately. And let us consider the oil first. When I first came here not quite a year ago it was my understanding that the biggest single problem facing the cottonseed oil industry was the cyclopropene acid problem. It is the cyclopropene acids in cottonseed oil that give the Halphen test that is generally regarded as characteristic of cottonseed oil. They also

produce pink egg whites in eggs from laying hens that have been fed cottonseed oil, or cottonseed meal that has more than about half a percent of residual cottonseed oil. They may also have other physiological activity. So, the cyclopropene acid problem was considered a very important one and we are spending more research effort on that than on any other single cottonseed oil problem. It has been very frustrating but we have been making progress. How much progress we may find out in a working conference on cyclopropenes which will be held on Wednesday and Thursday of this week. At that time people actively engaged in research on cyclopropenes all over the country, from Arizona, California, Texas, Michigan, and Illinois, as well as at the Southern Division, will spend 2 days here reporting their latest research findings and comparing notes.

In the Oilseed Crops Laboratory we have spent considerable time on analytical methodology, that is, on development of an analytical method to determine cyclopropene acids. There is only about half a percent of cyclopropene acid in cottonseed oil so any method for its determination has to be quite sensitive. Dr. Skau here has developed a method for determination of cyclopropenes in cottonseed oil that he feels is accurate to 0.01 percent. That is, he can distinguish 0.45 percent and 0.46 percent cyclopropenes in cottonseed oil. The method requires considerable skill and experience but you may be interested to know that a commercial testing firm has recently undertaken to use this method. I understand they are now prepared to conduct analyses for cyclopropenes in cottonseed oil on a fee basis. We are still not completely satisfied with the method and are trying to simplify it and to adapt it to a micro scale so that, for example, we will be able to determine the cyclopropene content of the residual lipids that we might extract from a cottonseed meal. I should say that we do not now have a method suitable for the determination of cyclopropenes in cottonseed meal. This is a problem that Dr. Skau and his group are working on right now. However, he has used the method he has developed for studying the cyclopropene content of the oil from 25 samples of cottonseed of different varieties and species. He found that the cyclopropene content of the oils from these 25 different cottonseed samples ranged from about 0.5 percent to a little more than 1 percent, as malvalic acid.

In another phase of our research on cyclopropenes we are trying to prepare some extremely pure cyclopropene acids for use as reference standards and to study the properties of the pure compounds. We have spent a great deal of effort here at the Southern Division on the preparation of a pure sample of any naturally occurring cyclopropene acid but we have never yet been able to obtain a sample that we could all agree was really pure--that is, at least 98 percent pure. It should not be that tough a problem but it has been.

Also, we are in the process of preparing a relatively large amount of concentrate of cyclopropenes from cottonseed oil that would be used for feeding tests with experimental animals, particularly chickens and rats. Our objective is to prepare 5 to 10 pounds of a concentrate that contains at least 50 percent cyclopropenes, which would mean a hundredfold increase over the 0.5 percent that is usually present in cottonseed oil. Most of the large scale work is being done in the Engineering and Development Laboratory but the Oilseed Crops Laboratory is cooperating closely in that work and participating in it. This work is supported by the Foundation for Cotton Research and Education, and I want to take this opportunity to express my appreciation for that support. In somewhat related work we have removed the nonsaponifiables from cottonseed oil, converted the oil to simple esters (the ethyl esters), and fractionated the esters into a fraction relatively richer in cyclopropenes and another relatively poorer in cyclopropenes. These we supplied to the Pharmacology Laboratory at the Western Division for rat feeding tests. Corn oil is being used as a control, and in one test Dr. DeEds is feeding rats the nonsaponifiables that were removed from the cottonseed oil. The reason for that is there is some evidence that some of the physiological activity that

has been attributed to cyclopropenes may not be due to the cyclopropenes at all. It may be due to something else. This work may help evaluate the relative physiological importance of cyclopropenes and other constituents.

Research is also under way in this laboratory on methods of inactivating cyclopropenes which includes inactivation by partial hydrogenation. Research on hydrogenation of cottonseed oil is being conducted in Mr. Feuge's group. We are doing additional work on cyclopropenes under contract. We have two contracts on cyclopropenes at the University of Illinois. One deals with the development of basic information as to the chemical and physical properties of cyclopropene fatty acids; the other deals primarily with the development of methods of eliminating or physiologically inactivating the cyclopropenes. We also have a Public Law (PL)-480 project with Dr. J. R. Vickery of CSIRO (Commonwealth Scientific and Industrial Research Organization) in Australia on the chemistry and biological effects of cyclopropenoid compounds that occur in cottonseed products. The project was initiated just about a month ago, in January 1964. Also, we are now planning a contract to be directed toward the development of a practical process for inactivation of cyclopropenes in cottonseed meal--that is for meal, not oil. This process may permit the feeding of cottonseed meal that contains considerable residual oil in it to laying hens without getting pink egg whites.

Much, but not all of our research on cottonseed oil, is related to the cyclopropene problem. We are still involved with our old friend gossypol as it relates to oil color and to the fixation of oil color in cottonseed oil. Some of that work is being done here in New Orleans. At least it was until about a month ago when the incumbent resigned. Additional research is being conducted by contract at the University of Tennessee under Dr. Shirley.

Research is also being conducted on the utilization of cottonseed oil. I have already mentioned that research on hydrogenation of cottonseed oil is being conducted in Mr. Feuge's group. His group is also looking into possible new edible uses. You have all heard of the acetoglycerides and cocoa butter-like fats that were developed by Mr. Feuge and his group. He has some other ideas for novel edible materials that can be prepared from cottonseed oil. Also, I should mention our work on fat emulsions for intravenous alimentation. That is a project that has been going on here in Mr. Feuge's group for a number of years. It is largely supported financially by the Office of the Surgeon General rather than by our own laboratory funds. In the course of that work we have developed what we believe is a suitable fat emulsion based on cottonseed oil.

A limited amount of research is underway both in this Laboratory and under contract directed toward industrial utilization of fatty acids that may be derived from cottonseed oil. Here in New Orleans, Dr. Skau's group is carrying on research on the preparation and evaluation of materials that may be used as plasticizers. At Purdue University we have a contract with Dr. Brown on hydroboration to produce useful products and cottonseed fatty acids are included in that contract. Also, we have a contract with the University of Arizona where Dr. Marvel is conducting research on the production of polymers. We are right now planning a contract with an industrial firm for research on the preparation of copolymers with ethylene. Here, too, it is planned to include cottonseed fatty acids in the contract.

Also, and finally, I should tell you that some research on the utilization of cottonseed oil is being done for us abroad with PL-480 funds. One project that was just completed in France involved the preparation of more than a hundred chemical derivatives of fatty acids. Some of these which are based on fatty acids derived from cottonseed oil have potential industrial utility. Another project I should mention, in England, is on the crystallization behavior of cottonseed oil as related to its glyceride composition.

Now, if we may leave cottonseed oil and turn to cottonseed meal, we are continuing research on the mixed solvent process for extraction of cottonseed with the mixed solvent (acetone-hexane-water) to develop information that is basic to the production of cottonseed meal and oil of the highest quality. Research is continuing too, in cooperation with others, on the nutritive properties of meals obtained by extraction of cottonseed with the mixed solvent. This includes tests with broilers and laying hens and with swine. A test with swine is now underway at Beltsville, Maryland. So far the mixed solvent meals have showed up very well indeed. But we are also trying to find out why some cottonseed meals are not as suitable for unrestricted feeding to swine. Among other things too we are studying the effect of gossypol and gossypol related materials as they may affect color of egg yolks and whites and other properties of eggs when they are fed to laying hens. This work is being done in cooperation with the Ralston Purina Company.

We are also conducting research on the amino acids of cottonseed in Dr. Frampton's group. This includes research on analytical methodology--the development of better methods for the determination of some of the amino acids. We are now in the process of determining the amino acid composition of individual proteins or protein fractions obtained from commercial cottonseed. I will plan to refer to this work again a little later.

That covers, although very briefly, the vast majority of our work on cottonseed oil and meal. But I would like, with your permission, to tell you very briefly about two other matters. You may remember I told you earlier that when I first came here not quite a year ago I understood that the most pressing problem was the cyclopropene problem. I had been here only a few weeks when the trout hepatoma problem broke into the news. We are involved in the trout hepatoma problem and are following it very closely. Most of the experimental work done at the Southern Division on the trout hepatoma problem has been done in Mr. Vix's Engineering and Development Laboratory and I hope he will tell you about that. But, I felt I should mention that the Oilseed Crops Laboratory has participated in this work and we are following it closely. Also, plans are well advanced for research on the possible role of cottonseed meal in the production of trout hepatoma with PL-480 funds in Italy. Dr. Pietro Ghittino in Turin is a recognized authority in this field. We are very fortunate in having this research done with PL-480 funds.

About a month after the trout hepatoma problem broke, the aflatoxin problem made news. The aflatoxin problem really started in 1960 when a new disease killed about 100,000 turkeys in England in a few months. In the unimaginative way scientists sometimes have, it was called Turkey "X" disease. The cause was quickly traced to a moldy Brazilian peanut meal used as an ingredient in the feed. The mold was identified as Aspergillus flavus, and it was found that some strains produce a highly toxic material. This toxic material was called aflatoxin, from Aspergillus flavus and toxin. Crude aflatoxin is a mixture of several different components and two of them, aflatoxin B and aflatoxin G, (from the blue and green fluorescence they exhibit when illuminated with ultraviolet light) are extremely toxic. Different animals exhibit widely different susceptibility. The duckling appears to be the most sensitive and only 28 micrograms will kill a 1 day old duckling. Put another way, that means that 1 ounce could kill a million ducklings.

Aflatoxin is also extremely carcinogenic. In the spring of 1963 it was announced that a domestic commercial peanut meal when fed to rats produced a high incidence of carcinomas. I should emphasize as strongly as I can that these are not edible products - they are used for feed for animals and not food for humans. We now know that purified aflatoxin when fed to trout will produce hepatomas even when fed to them in very small amounts. I do not want to say, or even to imply, that the trout hepatoma problem is related to the Turkey "X" disease. But Aspergillus flavus spores occur everywhere.

I am sure there are some in this room. Since the Oilseed Crops Laboratory is interested in both cottonseed and peanuts we have begun a research program dealing with molds and mold metabolites in cottonseed and peanuts. As of now we have three people working full time on that problem and one of them is working full time on cottonseed. We will probably expand that program. We are also planning a contract to study the limiting environmental conditions for the production of mycotoxins, especially on peanuts but much of the information developed would doubtless also be applicable to cottonseed and other agricultural products used for feed and food.

Now, finally, there is one last piece of work planned that I would like to mention. By way of introduction I would like to refer again to the gossypol problem. When this Laboratory began work on gossypol in the early 1940's it had already been known for a long time that there is a wide variation in the gossypol content of cottonseed. In the early 1920's there was established a general correlation of gossypol content and area of production in the United States. That is, the importance of environment was recognized. In the middle 1930's a Russian lady by the name of M. Smirnova showed, apparently for the first time, that there are varietal differences in gossypol of cottonseed. She concluded that both environmental and genetic factors independently affect the gossypol content of cottonseed. In the 1940's Dr. Charlotte Boatner in our Laboratory made a thorough study of the occurrence of gossypol in cottonseed, and in the late 1940's she concluded, and I will quote "all of the gossypol and gossypurpurin of cottonseed occurs in isolated pockets or glands." I believe the key word here is "all"; all the gossypol.... of cottonseed occurs in isolated pockets. Boatner and others in our Laboratory, notably Pons and Hoffpauir and T. H. Hopper analyzed various samples of cottonseed and, in general, confirmed Smirnova's results. Pons, Hoffpauir, and Hopper concluded that "both variety of cottonseed and the environment have a highly significant influence on the gossypol content of the kernels" and that "the gossypol content of the kernels may be a genetical characteristic". It is noteworthy that these investigators found the gossypol values for one variety of cottonseed, Acala 4-42, consistently low.

Then in 1954 there was discovered a completely glandless boll in a variety of Acala cottonseed. It was reported by McMichael who at that time proposed the use of the glandless boll as a marker for measuring the amount of natural crossing in cotton. By a glandless boll he meant "the complete absence of oil glands in the bolls". Put together Dr. Boatner's observation that all the gossypol of cottonseed occurs in glands and McMichael's glandless bolls marked by the complete absence of oil glands and you have the beginning of the program that has resulted in the development of cottonseed that is free of gossypol--or practically so. This is what we all know now as glandless cottonseed. Incidentally many of the gossypol analyses in those days were made in our Laboratory.

That was a long introduction to what I really wanted to tell you about. Consideration is now being given to a grant in a related area dealing with possible improvement in the protein or oil quality of cottonseed. We conduct research in several ways: in house here in New Orleans, cooperatively with others, for example, with State Experiment Stations; by contract, in foreign countries with PL-480 funds; and finally by grants. Grants give the investigator much more freedom as to how he conducts his research than is possible with contracts. We have had authority to make research grants for a number of years but we have not used that authority until this year. In the Oilseed Crops Laboratory we are now planning our first grant; that is, to investigate the possibility of genetic improvement in the protein or oil quality of cottonseed--not just by breeding out an undesirable component like gossypol, although we probably would not mind breeding out cyclopropenes, but by breeding in a better material; for example, a better protein. The experimental background for the real possibility of breeding cottonseed for

better protein is not as clear as it was for gossypol, but geneticists indicate that possibilities exist for genetically altering amino acid ratios in cottonseed protein. If that could be done, it might be possible to develop a cottonseed with a higher proportion of the desirable amino acid lysine in cottonseed protein. That is the purpose of this proposed grant--to see if it is possible by genetic means to increase the quality of the protein and of the oil of cottonseed.

CURRENT COTTONSEED RESEARCH AT THE SOUTHERN DIVISION

in the
Engineering and Development Laboratory
by
Henry L. E. Vix
Southern Utilization Research and Development Division

The Engineering and Development Laboratory participates in research on all commodities under investigation at the Southern Utilization Research and Development Division. My remarks will be concerned only with the research on cottonseed.

The current programs of research and development work on cottonseed in the Engineering and Development Laboratory are:

1. Concentration and purification of cyclopropenoid fatty acids.
2. Special extractions of cottonseed meals for the trout hepatoma problem.
3. Acetone-hexane-water extraction of cottonseed meats.
4. Bleaching off-colored cottonseed oils with activated alumina.
5. Improved cotton batting through mechanical-chemical treatment.

The first two programs are principally service in nature, but are of extreme importance to the continuance of a stable utilization of oil and meal products from cottonseed. Though cottonseed products have been marketed successfully for many years even today some questions still arise concerning whether cottonseed products, such as the meal, may under certain conditions of preparation have some adverse physiological effect on man and animal. Hence, we are requested to do research work to isolate sizeable quantities of some of the minor constituents from cottonseed and cottonseed products so that they can be used in special and specific experiments by various investigators. We accept these requests for such preparations because we feel that this is one way that our efforts can be advantageously used to help maintain the status and importance of the cottonseed industry.

The other three programs of research all deal with process developments to produce improved products from cottonseed. For all three, we have been in close association with chemical manufacturers, equipment manufacturers, and industry representatives to gain knowledge and technical and engineering information required. Such contacts have aided us in achieving promising results on each of these three process developments.

Concentration and Purification of Cyclopropenoid

Fatty Acids. (Project Leaders - E. L. D'Aquin and W. Landmann) The Engineering and Development Laboratory has been assigned the task of preparing methyl esters of cottonseed oils with the highest percentage of Cyclopropenoid Fatty Acid (CPA) that can be obtained in a practical manner. The need for such material was mentioned by Dr. Goldblatt in his presentation.

The method recommended was low-temperature (-68° C.) crystallization of methyl esters from methyl alcohol solution to remove saturated and unsaturated straight-chain fatty acid esters as crystallized products from methyl malvalate which remains in solution. Powdered Dry Ice is introduced directly with the methyl alcohol solution of the methyl esters to achieve the low temperature (-68° C.) required for the crystallization. Necessary batch pilot plant equipment has been designed, constructed, and placed in operation for this work. An immersion,

filter leaf with about 1.8 sq. ft. of filter area is used to remove the filtrate from the crystallized nonmalvalic constituents.

In the first crystallization the esters in filtrate contain 4 percent of malvalic acid, representing an eightfold increase from original cottonseed oil that contains about 0.5 percent malvalic acid. In the second crystallization the malvalic content of the esters is doubled to about 8 percent. Further crystallizations are to be studied, but the exact procedure details are not yet firm.

Operations through the second crystallization have been carried out in the pilot plant, and, as a result to date, we have prepared over 4,000 grams (about 9 pounds) of 8 percent concentrate of malvalic acid. The anticipated requirement for CPA concentrates has been estimated at about 7 pounds of about 50 percent concentrate of malvalic esters.

Special Extractions of Cottonseed Meals for the Trout Hepatoma Problem

(Project Leader - H. K. Gardner). This problem became suddenly significant last year. Work is underway to determine whether cottonseed meal is a causative factor. Dr. Goldblatt also mentioned some of the investigations underway with cottonseed. At the request of the National Cottonseed Products Association, five lots of commercial cottonseed meal were extracted in our batch extraction pilot plant by a special procedure outlined by Dr. Goldblatt of SU, Dr. Richard A. Phelps, NCPA, Dr. John E. Halver, Western Fish and Wildlife Service, Department of the Interior, Cook, Washington, and Dr. Gillis La Roche, University of California. The procedure called for each meal to be first extracted with a mixture of diethyl ether-ethanol (130 gallons) followed by a second extraction with a mixture of diethyl ether-ethyl acetate (150 gallons). For each meal the extracts from the two solvent mixtures were carefully concentrated at a temperature not exceeding 104° F. (40° C.) and then combined. The five meals were extracted on schedule. Each required careful supervision due to the hazardous nature of the solvent involved. The five extracts were promptly forwarded to Dr. Halver for his trout hepatoma studies.

Acetone-Hexane-Water Extraction of Cottonseed Meats

(Project Leader - E. A. Gastrock). Details of this research will be reported by Mr. Gastrock in his presentation entitled "Progress Report on the Hexane-Acetone-Water Extraction of Cottonseed Meal."

Based on pilot plant results good progress has been made on this development. We now have a promising and demonstrable process.

Bleaching of Off-Colored Cottonseed Oils with Activated Alumina

(Project Leader - P. H. Eaves). Like the acetone-hexane-water extraction of cottonseed meal, this development is the outgrowth of research initiated by the Oilseed Crops Laboratory. It has been successfully developed first on a bench scale, then a pilot plant scale, and now is under evaluation by industry. The basic process developed was presented by Mr. Paul H. Eaves at the last (Twelfth) Cottonseed Processing Clinic. Cost analyses made by Mr. K. M. Decossas, Engineering and Development Laboratory, indicate that the cost of alumina bleaching as presently developed would approximate re-refining and earth bleaching for current commercial oil refining operations.

Some recent and important achievements were:

1. Based on necessary specifications established through our research, industry has developed an activated alumina suitable for use and at a price (10.5 cents per pound, carload lots) which makes the process feasible for commercial scale of operations. Over 25 samples of activated alumina have been submitted by industry. Many of them

were experimental products prepared especially for our use. Among those submitted were a number which proved very efficient as oil bleaching agents. Such activated alumina products could lead to a solution of cottonseed oil color problems when their costs become sufficiently low enough for commercial use.

2. Activated alumina only absorbs the red color from the refined oils. Its removal unmasks the green color which is always present in varying degrees in cottonseed oil. It was found that the inclusion of activated carbon (0.15 percent, based on weight of oil) with the activated alumina removes the green color efficiently.

3. Filtering finely divided alumina from bleached oils proved to be a major operational problem. It was found that coarse particles resulted in good filtration rate but yielded poor bleaching of the refined cottonseed oil while fine particles produced good bleaching results but slow filtration rates. By adjusting particle size distribution of the activated alumina in the range of 15 to 16 microns both optimum bleaching and filtration rates were achieved.

4. For good bleaching action activated alumina must have a moisture content of about 10 percent. This problem was solved by mixing alumina with water to form thick slurry prior to its addition to the oil. This procedure greatly simplifies the addition of alumina to the oil. It also enhances the bleaching efficiency and for very finely divided alumina improves the filtration rate.

The alumina bleaching method has now been developed into a practical batch pilot plant process which has potential as a commercial means for off-colored cottonseed oil.

Improved Cotton Batting Through Mechanical-Chemical Treatment

(Project Leader - N. B. Knoepfler). This is a cooperative project jointly sponsored by the Textile Waste Association, the National Cottonseed Products Association, the National Cotton Batting Institute, the Foundation for Cotton Research and Education, and the Southern Utilization Research and Development Division. Research was initiated in 1961 and was directed at finding a means of improving the performance of cotton batting to enable it to better compete with synthetic foams in the cushioning markets (furniture, automotive, and mattress). Approximately 60 percent first-cut linters and 40 percent cotton textile waste are used in conventional cotton batting.

Mr. N. B. Knoepfler reported at the last (Twelfth) Cottonseed Processing Clinic the results of our initial research and outlined the processing operations developed to produce an improved cotton batting through the application of a resin-latex treatment. We have further improved the process and product through our research efforts of the past year. We have developed a good product and a workable process.

In comparison with current conventional cotton batting, our improved cotton batting possesses:

1. Textile structure with better thickness retention.
2. More resilence in both the individual and bulk fibers.
3. Improved cohesiveness, structural and dimensional stability.
4. Higher bulk with less weight.
5. Greater and more rapid recovery from deformation loading.
6. Better mold and shaping characteristics.
7. Better color.

In addition, our batting product retains to a large degree much of the desirable properties of current conventional cotton batting, such as initial softness, good heat transfer, minimum effect of temperature and humidity in service.

We are currently developing other chemical-mechanical approaches to achieve further improvements, particularly in maintaining much of cotton's initial softness and yet achieve a very high degree of initial resiliency.

We have had over 200 contacts with various segments of the industry. Such contacts have kept us busy, but they have been a great help in realizing the progress to date. To further aid us in achieving our ultimate product we had recently a workshop meeting, with over 100 people in attendance. There were representatives from the three principal markets for cotton batting, representatives from chemical industries that manufacture resins and latexes, and from industrial concerns that manufacture the necessary equipment used for the various operations in our process. Also, recently in Washington,D. C., through the efforts of the National Cotton Council, we met with technical representatives of a dozen chemical industries to obtain their assistance in designing improved resins and latexes, particularly applicable for our process.

Several industries are currently evaluating our process on a pilot plant scale, and have produced some very good products. The automotive, mattress, and furniture industries are currently evaluating some of the products produced by us as well as those produced on pilot plant scale by the batting industry.

(Note: The movie made by the National Cotton Batting Institute was shown at this Clinic meeting. This movie shows the process developed at the Southern Division pilot plant operations of this process by industry, and clearly delineates the various improved properties of the resin-latex treated cotton batting.)

REVIEW OF PROTEIN CONCENTRATES

by
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National Cottonseed Products Association

The primary purpose of this paper is to review the information presented at the "Conference on Cottonseed Protein Concentrates" which was held in New Orleans on January 15-17, 1964. Obviously, the allotted time does not permit a detailed review of the data which required almost 3 full days of crowded presentations at that conference. It is equally obvious that the great diversity of subject matter covered gives good cause to label anyone as an egotist who attempts to summarize it into detailed conclusions. Therefore, it would appear desirable to lay down some "ground rules" for our function here today and to prescribe some limits beyond which we shall not attempt to go.

We shall attempt to convey to you the spirit and the degree of progress represented in the conference. The conclusions expressed here shall be largely those of the conference participants. It shall not be our function to attest to the logic of these conclusions nor to argue with the soundness of the data presented to support them. At the same time, we shall not hesitate to cite other studies not fully reported at the conference when there appears to be an especial need to do so.

This was truly an international conference from the standpoints of participation, needs, and opportunities. In no sense was it merely a provincial discussion of the problems and advantages of cottonseed protein. The conference chairman, Dr. A. M. Altschul, in his attempt to summarize the findings of the conference at its close, appropriately called attention to the fact that foreign scientists who had attended in the past largely to listen were among the leaders in presenting new data. Perhaps of even more significance was his interpretation that the direction of the deliverations had departed from the limited scope of consideration of protein source (cottonseed, soybean, animal, etc.) and appeared to be traveling the more sophisticated pathway of the evolvement, characteristics, and function of the total protein community.

The overriding theme of the conference was that of the usefulness of vegetable protein in general and cottonseed protein in particular in the human dietary. Necessarily, the range of information presented extended from fundamental botany to human psychology with intermediate points at chemistry, genetics, nutrition, economics, and animal physiology.

Frank Barlow and Wayne Bitting (1) documented the world-wide deficiency of protein and the lack of even distribution between countries in various stages of development. Their data showed that domestic production in relationship to need, declines as one moves from the rich to poor countries. Further, commercial imports supplied a smaller portion of needs in poor than in rich countries. On the other hand, special program imports, such as PL-480, largely made up the difference from lack of commercial imports, leaving deficient national production the predominant factor in lack of availability. They concluded that the rapidly developing countries show good promise of being able to overcome deficiencies through increased domestic production and increasing ability to purchase commercial imports. On the other hand, they suggested that slowly developing countries, with their rising populations, appear to have little opportunity to close the nutritional gap except through increasing levels of special program imports.

Of interest is their conclusion that the most important commercial markets for specialty or highly processed food products are in the higher income countries, but that there appears to be a need there for low-cost proteins to meet the needs of the lower income bracket in these well developed countries. They concluded that the rapidly developing countries probably offer the most attractive commercial market for high protein concentrates because of their smaller dependence upon animal proteins.

Max Milner of United Nations (2) spoke specifically to the point of assisting underdeveloped countries to meet their protein requirements through the use of low-cost proteins produced within those countries. He pointed out that cottonseed is the most widely distributed oilseed in tropical and subtropical areas while soybeans are not widely grown in areas of protein deficiency, with the exception of Mainland China. He reported that their dependence upon the conviction that nutritional quality and feeding safety were sufficient reasons to assume UNICEF programs in use of locally produced oilseed meals had resulted in partial failure. Lack of understanding of food value, how to formulate it into an acceptable product, and local resistance to change must be overcome in any successful program. He especially emphasized the difficulty in having available at low cost a cottonseed protein of uniform high quality and expressed doubt that many oil mills could cope with the complex technology involved. He urged a tremendously broadened research attack upon oilseed protein materials to make possible their fullest use in the human dietary and expressed the thought that the urgency of world food problems will not condone the slow and empirical evolvement of food trends as they have occurred in the past.

Harold L. Wilcke (3) and Garlon A. Harper (4) presented data which show that U. S. production of cottonseed has remained static since World War II, the domestic production of cottonseed meal has been largely fed to livestock and poultry with an increasing amount being channeled through feed manufacturers, and that type of processing has shifted from 95 percent hydraulic in 1945-46 to 59 percent screw press, 38 percent solvent extraction, and only 3 percent hydraulic pressing in 1962-63. Wilcke proposed that animal and poultry feeds cannot continue to absorb, at favorable price levels, more and more plant proteins and that human consumption appears to offer the brightest prospect for future expansion.

Results of fundamental studies of the structure of the cottonseed and its component parts were reported by J. R. Wall (5), Lawrence Yatsu (6), and Wilda Martinez (7). In his summary statement at the close of the conference, Dr. Altschul cited the urgent need for expansion of such basic work to provide the fundamental knowledge on which future expansion of processing and utilization technology might come. The work of Martinez which indicates that different amino acid patterns are to be found in various sections of the seed kernel give a glimpse of the significance of the work and the meaning of Dr. Altschul's comment.

A series of papers by Carl M. Lyman (8), Vernon L. Frampton (9), and Richard A. Phelps (10) reviewed chemical and biological data obtained on the constituents of cottonseed and their effects upon the biological value of cottonseed protein. The work of Lyman confirms that pigment gland gossypol and gossypol-like compounds have a higher degree of toxicity than does pure isolated gossypol and he proposed that a newly discovered gossypol-like compound he has designated as gossyverdurin may be partially or completely responsible. He reported that gossypol tolerance of swine is increased when the diet contains high-quality fish meal or ferrous iron in the monohydrate form. He also called attention to earlier reported studies which establish that boiling cottonseed meal reduces the amount of available gossypol without a significant reduction in lysine availability. Frampton reported that the statistical techniques employed to evaluate growth data from a large collaborative broiler experiment did not reveal any significant correlation between dietary free gossypol levels and growth

response. We would caution against the interpretation that gossypol does not depress growth when fed in sufficiently high levels. Other investigators have noted such an effect and we believe the conditions of the collaborative feeding trials and the data obtained did not lend themselves to the type of evaluation attempted. His finding that lysine was a major contributing factor to biological value of the cottonseed meals fed is in agreement with the results of other studies. In his review of the role of cyclopropenoid fatty acids upon the utilization of cottonseed protein concentrates, Phelps concluded that the available data indicate that, when fed at relatively high level, these minor fatty acids induce undesirable effects including (a) changes in membrane permeability and/or yolk structure, (b) altered ratios of fatty acids in deposited fat, (c) increased chick embryo mortality, and (d) retarded development of reproductive organs. His review suggests that little evidence is available to indicate that these effects are produced when practical levels of cottonseed products are fed; however, dietary levels of residual cottonseed lipids must be low to insure freedom from pink discoloration of egg whites. Phelps discussed recent studies which indicate that the pink discoloration and changed fatty acid ratio may be induced by a fraction of the lipids without the cyclopropene configuration. Clarifying research is needed.

Five papers were presented to report general and/or specific attempts to use cottonseed meal and/or other protein sources in the development of new protein foods. Wilcke (3) aptly cited the need for both basic and applied research of the most imaginative kind to make vegetable proteins achieve their full potential in the diets of mankind. He directed attention to five forms of soybean protein which are available commercially, including soy flours, isolated soy proteins, less purified soybean concentrate, the water soluble Gelsoy, and the new physical forms of spun soybean protein which may be made into products of desired texture and physical form. He called attention to the almost unlimited opportunities these developments bring to the food technologist to control such important factors as nutritive value, texture, flavor and eye appeal and reported that a large amount of such utilization research by many companies is in progress. His prediction that the plant protein industry will unquestionably assume a broader and more significant role in total food production than that of its present contribution to food through animal agriculture would appear to be well supported.

H. W. Bruins (11) reported encouraging success by the Quaker Oats Company in the manufacture and marketing of an Incaparina product in Colombia. The degree of progress apparently being achieved would suggest that the use of such low-cost vegetable protein foods need not necessarily be limited to national or international welfare organizations. Quite dramatic confirmation of this premise is found in the report of P. H. Hind (12) on the development and merchandising of "ProNutro" a high protein food in South Africa. Composed of skimmed milk, peanut and soybean protein, fish flour, food yeast, wheat germ, bonemeal iron, iodized salt, sugar, and B vitamins, the mixture was formulated to provide the amino acid pattern and biological value of fresh skimmed milk at a cost of slightly more than one cent per day per child in areas where milk is scarce and expensive. Developed as a profit enterprise by this private company, the product was subjected to a comprehensive program of market research, merchandising, public relations, and advertising with results which create considerable optimism. Beginning with the sale of 72 tons in 1962, consumption is expected to reach 2,800 tons for the year ending June 1964.

Ricardo Bressani (13) reported a tremendous volume of research conducted on the technological aspects of Incaparina, the low-cost protein food which contains 38 percent cottonseed meal. Faced with uncertain availability of cottonseed flour of low cost which would meet standards that had been established, Dr. Bressani extensively studied seed variations and evolved guidelines for processing conditions which would effectively reduce free gossypol content and maintain acceptable pro-

tein quality. His data led him to the interesting conclusions that prepress solvent extraction with a normal amount of hulls included with the meats gave lower free gossypol and higher available lysine values than did processing hull-free meats. Of interest, also, was Bressani's report that his data indicate that nutritive value of the meal is higher when residual fat is low. It appears that these findings should be the subject of further study to determine whether they can be confirmed and, if so, elucidation of the cause sought. Using dogs as test animals, it was found that the total amount of ingested gossypol was recovered in the feces, but feces free gossypol was about three times higher than ingested amount. Additions of calcium and iron separately to the Incaparina mixture each reduced free gossypol and the additives appeared to have a complementary effect when added together.

Antonio Bacigalupo (14) reported Peruvian studies with the preparation of a reprocessed prepressed, solvent extracted cottonseed meal of high quality. Free gossypol in the meal was reduced by moist autoclaving or by adding water and drying at elevated temperatures. He found that under the conditions of his studies neither nitrogen solubility or epsilon free amino lysine values were always correlated with biological value.

Efforts to improve the biological value of cottonseed protein through breeding or the use of processing solvents other than commercial hexane were discussed by Charles F. Lewis (15), A. B. Watts (16), V. L. Frampton (17), Henry L. E. Vix (18), Carmelo Vaccarino (19), and Stanley P. Clark (20). Lewis reported that extensive studies had failed to establish any good cause to believe that commercial glandless varieties of cotton of high agronomic superiority cannot be made available to the cotton grower within the reasonable future. He proposed that realization of this objective rests upon the integration of the success of the breeder into the economic system which involves the seedsman, grower, oil mill, and the consumers of cottonseed products. Watts discussed the results of feeding trials which caused him to conclude that glandless cottonseed meals can be produced, without the use of complicated solvent systems, which are superior to present high-quality commercially produced glanded cottonseed meal and fully equal in feeding value to soybean meal.

Frampton and Vix described the development of a mixed solvent of hexane, acetone, and water which may be used to process cottonseed meal having low free and total gossypol and high epsilon free amino lysine values. Vix proposed that the process is now developed sufficiently for serious consideration for commercial trial, especially in areas highly dependent upon cottonseed meal as a source of protein for nonruminant animals. Frampton reviewed biological data which led him to the conclusion that meal produced by the mixed solvent process has highly superior biological value. Data presented by Watts suggest that the mixed solvent meal may be fully equal in biological value to soybean meal and glandless cottonseed meal when used as the major source of supplemental protein in practical type diets fed broilers. Relatively little data are available on the acceptability of either mixed solvent or glandless cottonseed meal as a source of protein for swine; however, Frampton presented preliminary data from a current trial with pigs at the Agricultural Research Center, Beltsville, Md., which show the mixed solvent meal diet to be leading both soybean meal and commercial cottonseed meals in growth response.

Carmelo Vaccarino described a patented processing procedure being used by his company in Italy to produce a superior cottonseed meal through the use of acetone as a solvent for extracting oil from cottonseed. Free gossypol values were reported to be in the range of 0.01 to 0.03 percent and total gossypol ranged from 0.26 to 0.54 percent, which is comparable to the values expected from the use of mixed solvent. When prepressing was employed epsilon free amino lysine values were reported to be in the area of 3.25 grams/16 grams of nitrogen--about the same as high quality prepress solvent extracted meals in the United States. With the use of reduced

temperatures in direct solvent extraction with acetone, values above 4.0 grams/16 grams nitrogen were obtained. Vaccarino reported that feeding trials with rats, swine, and chicks demonstrated a high biological value for the acetone extracted cottonseed meal. Clark proposed numerous questions on processing details which appear to need to be resolved before oil mill management arrives at a decision to convert to the Vaccarino process. He stressed that heat requirements and acetone cost may increase operation costs to the extent that serious consideration must be given to whether the acetone-extracted meal will demand a premium adequate to justify the conversion.

John A. Price (21) reviewed the availability of new solvents for oil extraction and meal quality improvement. He indicated that new developments in the future are more likely to be in the use of mixtures of solvents rather than the discovery of a new individual solvent. This suggests that commercial hexane still appears to be the best compromise when only one solvent is employed. We find some difficulty in agreeing with his assumption that the only undesirable characteristic of hexane is its flammability because it extracts gossypol from the meal quite poorly.

Leo Friedman (22) currently of the Department of Nutrition and Food Science at MIT and formerly a member of the research staff of the Food and Drug Administration eloquently reviewed a long list of adventitious toxic factors of concern to the producers and consumers of protein concentrates. We recommend highly that the practical processor of any food or feed product make himself familiar with the subject matter discussed by Dr. Friedman. The significance of the numerous hazards appears overpowering at first consideration. However, as Dr. Friedman points out, these problems have existed since Biblical times and the scientific community is rapidly developing the knowledge and skills to insure appropriate solutions. As is so frequently possible in such brief reviews, incorrect impressions can be created by the necessary brevity of the review. We feel that Dr. Friedman may have given too much weight to California reports intended to incriminate cottonseed meal as a factor in trout hepatoma without adequate coverage of other research which is in conflict with such a premise.

In summary, we feel completely justified in the conclusion that the Conference on Cottonseed Protein Concentrates heralds a new era in the use of vegetable proteins. It brought together a wide diversity of interests and provided stimulation for the development of needed thinking and possible new approaches which will guide future activities in the most desirable pathways. The developments reported in the conference discussions emphasize that the extent to which cottonseed protein will share in new developments will depend to a large degree upon the quality of the research effort devoted to its special problems.

As a representative of the cottonseed crushing industry, I am pleased to have this added opportunity to express our appreciation that the Agricultural Research Service of the United States Department of Agriculture and the United Nations Children's Fund group joined with the National Cottonseed Products Association in sponsorship of the conference. Also, very special appreciation is due Dr. Aaron M. Altschul whose intense interest and devotion made him the key factor in the successful organization and conduct of the conference.

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AVAILABILITY OF EDIBLE SEED PROTEIN CONCENTRATES

PRESENT AND FUTURE

By
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Introduction

In recent years I have had several occasions to prepare statements on this subject for international conferences concerned with the question of protein supplies. And recently I completed a book which deals in detail, among other things, with the question of world protein supply. I should like today to present to you some of the issues involved. To some of you this will be old hat, and perhaps to others will be of lesser interest than some subjects much closer to home. But I hope that this presentation of a broad view may contain some aspects of interest to everyone.

Some who heard me discuss these matters say that I am 100 years ahead of the time; this would indeed be disastrous. I would hope that I am no more than 5 to 20 years ahead, if I am ahead at all. I will let you decide which of the diagnoses is the more correct.

Because of the limitations in time I will not review for you the problem of world protein supply and the arguments for serious consideration of seed proteins for direct consumption by humans. Suffice to say that throughout the world serious consideration is being given to this aspect, and strong arguments are appearing for thorough consideration of the vegetable sources of proteins as a more important part of the human dietary of protein concentrates.

Criteria of an Acceptable Protein Concentrate

Taking in its barest nutritional sense, a suitable protein concentrate must supplement a diet based on cereals; this is done well by the conventional protein concentrates from animal sources. Animal sources of protein have the virtue that the concentration is high and the protein is of good quality; none among the common sources contain less than 22 percent protein and most have more. Such sources provide additional trace nutrients and are free of harmful ingredients because the animal acts as an efficient filter removing toxic and interfering materials from its own food. Moreover, animal sources of protein are flavory and delectable to the palate; they provide a basis for interest in a meal. This then is our ultimate objective when we wish to consider protein concentrates from seeds, which presumably are cheaper than the conventional animal concentrates but which have been thus far utilized only to a limited extent in human food in western society.

Potential Quantities of Seed Protein Concentrates

The major source of proteins for humans and animals are the cereal grains--over 100 million metric tons of protein from cereals is available annually. Oilseeds have the potential of furnishing over 20 million tons of protein annually; to this can be added the potential from legumes exclusive of the soybean and the peanut. The amount of protein available from oilseeds and legumes exceeds that available from animal sources.

Status of Protein Concentrates from Oilseeds

Let us begin with the simplest kinds of protein concentrates made by the simplest and minimum number of operations, and hence the cheapest.

a. Soybeans. The soybean is unique among plant sources of proteins in that it is a protein concentrate as it stands. Soybeans contain 40 percent or more protein; hence, any edible product of the soybean is already a protein concentrate. Full-fat soy flours (about 43 percent protein and 23 percent lipid) are components of baby foods and other food preparations; improvements in the methods of manufacture by extrusion have recently been reported. The major problem is that the raw soybean contain inhibitors of several sorts. Some of them have been identified as hemagglutinins and trypsin inhibitors; there are probably other inhibitors not yet identified. Fortunately, the inhibitors, as far as we know, are destroyed or inactivated by heat. Hence, merely the operation of cleaning, dehulling, and cooking can provide a product which is satisfactory. There may be other problems with full-fat flours, such as rancidity on storage, which may need better solution.

As it now stands, this is the simplest kind of protein concentrate made by the simplest procedures. It obviously has a wide range of possibilities.

Full-fat soyflour is a product of modern technology; so is defatted soyflour and this is cheaper than the full-fat product because of the additional credit for the economic value of the oil. At one time, oil was the major economic product of the soybean; now the protein is the major economic product. Now the great poultry industry in the United States is based on corn and soybean protein, and this same pattern holds for modern poultry industries in other countries.

The 50 percent soy-protein concentrate made suitable for sensitive monogastric animals is equally suitable for humans provided that proper conditions of cleanliness and handling accompany its manufacture. Soybean proteins are a good source of lysine but are low in methionine; diets overwhelming in soybean content could be deficient in methionine. But proper blending with other ingredients and, if necessary, supplementation with methionine or its analogue, make satisfactory diets from cereal and soy protein possible.

In the Orient, probably a majority of the soybean protein goes into human foods as sprouted beans, in fermented products such as miso, or in curds such as tofu. Of the soybean protein manufactured in the United States, probably not more than 10 percent goes for human foods.

b. Cottonseed. Cottonseed is next to soybean in amount produced. Cottonseed can be grown in the tropics, hence it is indigenous to many areas where there now is protein deficiency.

Utilization of cottonseed for monogastric animals and humans is a development of our generation. Cottonseed cannot be considered as a natural protein concentrate, but becomes one when the oil is removed. It is the virtue of an oilseed that the mere removal of oil by mechanical or solvent means converts an otherwise fair source of protein into a concentrate containing over 50 percent protein. It is possible to produce a cottonseed protein concentrate containing 55 percent protein by reducing the lipid to less than 1 percent and by removing most of the seed coat. This alone, however, does not convert it into a form suitable for monogastric animals and humans. Cottonseed does not seem to contain the hemagglutinins and trypsin inhibitors of the soybean, but as you well know cottonseed contains gossypol. Gossypol is reactive chemically once it is removed from its location in the so-called pigment glands. It will react with free amino groups on proteins and with amine groups of other natural constituents of the seeds. It will also no doubt react with free amino groups of the tissues of animals which ingest it, although we do not know the mechanism of the toxicity of gossypol to monogastric animals. For some reason, probably because of the complex nature of the digestive process, ruminant animals have been able to tolerate raw cottonseed and gossypol. The exact role of gossypol and its combination

products in cottonseed protein concentrates is still far from being known, and there are differences of opinion about the relative importance of the gossypol in the finished product as compared, for example, to the role of the lysine content.

In any event, conditions of processing and properties of the final product have been defined so that cottonseed protein concentrates can be manufactured and used safely for monogastric animals and even for humans. Careful processing of cottonseed by screw-pressing or by prepressing followed by solvent extraction has produced products which have been fed in hundreds of thousands of tons annually to poultry and swine. Flours produced under the same type of conditions have been the major ingredient of Incaparina fed to children and has proven successful as a source of protein, devoid of toxic reactions.

On the basis of its analysis, it contains 4.4 percent lysine, cottonseed should not be limited in this amino acid, but in fact cottonseed products are. Lysine is easily rendered unavailable even though it is not destroyed during processing. This damage can be minimized by proper processing conditions.

Processing by conventional equipment is obviously a compromise, but it is a useful one; it would be better, of course, to remove completely all of the gossypol and retain completely all of the available lysine. There are some new approaches to this problem which include extraction by mixtures of solvents, such as the hexane-acetone-water mixture being developed at this Laboratory, or by extraction with acetone which has been done by Vaccarino. One might also consider processes for isolation of cottonseed protein under conditions for removal of most of the gossypol or even the selective chemical destruction of gossypol. Finally there is an extensive program of breeding a gland-free cottonseed which will, of course, eliminate completely the gossypol problem. All of this was discussed in detail at a recent meeting on Cottonseed Protein Concentrates held in New Orleans in January 1964.

c. Groundnuts or Peanuts. Groundnuts or peanuts from the very first have a record as a human food. They are widely eaten either roasted or boiled and as a popular ingredient of confections. Peanut butter is a popular spread. The peanut is not a protein concentrate but it is easily converted into one by removal of oil. The peanut as far as we know does not contain the toxic factors in the soybean. There is, however, the possibility that raw, oil-free peanut material is improved by heat-treatment. A peanut protein concentrate containing 50 to 55 percent protein, made simply by removal of the lipid, is a natural condidate for a protein-rich source. There are numerous instances in the literature of peanut products being fed to children to overcome kwashiorkor, protein deficiency disease. Peanut is deficient both in lysine and methionine, and possibly threonine. It can be rendered more deficient at least in lysine by excessive heat-treatment. Therefore, although all-vegetable combinations containing peanuts can be made nutritionally adequate by supplementation, it is much better to use peanut protein together with animal protein such as fish flour or dry milk solids rather than in an all-vegetable protein mixture. This does not in any way reduce the value of peanut protein concentrates because, even so, their inclusion can reduce the requirement for the more expensive animal protein.

Possibilities and Limitations of Protein Concentrates

Up to this point we have discussed protein concentrates on the basis of commodities. Let us now go back and look at them generally from another viewpoint.

a. Position of Flours. The greatest virtue of the low-lipid flours is their low cost. They are made by simple processes and the other product is a high-priced ingredient from oil. Obviously as they now stand, they could be more extensively used. In most instances it is a matter of education of the processor and consumer. It has often been stated that one of the difficulties with

oilseed protein concentrates is that these are also fed to animals. Therefore, there is the psychological disadvantage of presenting an animal food to humans. We recognize that this is real but we reject it as a valid argument since it can be clearly demonstrated that a separate plant is required to produce products suitable for human consumption. This situation has sometimes been confused by referring to the products from oilseeds as oilseed cakes or oilseed residues, inferring things more suited for fertilizer and animal outlets. It would be much simpler if we refer to these as protein concentrates or isolated proteins, whichever they are.

The intrinsic nutritive value of oilseed protein concentrates is not a problem once the limitations of the individual products are recognized and once they are incorporated into mixtures to produce balanced protein foods. Perhaps the greatest barrier to more widespread exploitation of their potential as a flour lies in the lack of constancy of the product and in the inadequacy of present methods of recognizing quality and content of deleterious or toxic factors. It can be easily shown that cottonseed, peanut, and soybean concentrates vary enormously in their nutritive value depending on how they were made even though their protein content is the same. This is owing in part, mentioned earlier, to the differential reduction of amino acid availability during processing. In order to acquire constancy of product it is, therefore, necessary to be able to describe a suitable protein concentrate by simple chemical tests. In some instances we are beginning to approach this capability. Carpenter at Cambridge and Woodham and the late Duckworth at the Rowett Institute were able to relate variability and nutritive value of fish meals to destruction and availability of lysine. Mauron showed that the same sort of measure can differentiate between overheated and well-dried milk powders. Frampton and his associates were able to show the same sort of relationship for cottonseed protein concentrates. In a few instances this was shown for peanut products.

Thus for a similar sort of measure has not been found for soybeans. Since they have more than enough lysine, and since lysine in the soybean is more stable to destruction during processing, a measure of available lysine does not tell the story. Numerous empirical measures have been tried, but they are only suitable for a limited range of conditions. It is quite possible that a measure of changes in sulphur amino acids would be the one to show differences between various soybean products. It is reported that in some instances 80 percent of the cystine in soybeans is destroyed in processing. Methionine, too, is a reactive amino acid, much more reactive than had been considered; chemical reactions are now available for studying the state of methionine and cystine; research in this area should prove profitable indeed.

There is also needed a measure of destruction of toxic materials during processing. We have already mentioned the measure of free gossypol which seems to be satisfactory enough for describing the situation in cottonseed. The absence of trypsin inhibitor has served as a guide for adequate treatment of soybeans, and may be sufficient. I do not believe, however, that the soybean processors are satisfied with this as the ultimate means of determining the complete removal or destruction of undesirable components. Nor are the cottonseed users satisfied completely with the gossypol measurements.

Recently attention has been directed to the presence in oilseed products of other toxic materials which I shall choose to call adventitious. These are not intrinsic in the product, but they are a result either of poor storage, harvesting, and processing conditions, or of improper treatment with pesticides. I would only mention the various X diseases reported in livestock. Some have occurred in cattle fed soybean products extracted with chlorinated solvents; and this has later shown to be the result of reaction with a sulphur amino acid in the protein to produce a toxin. There have been instances of the killing of cattle fed cottonseed meal which have been related to chlorinated naph-

thalene in the grease in the pelleting machines. There have been instances of deaths of animals fed meals supplemented with certain fatty materials which have been damaged by overheating.

Heat-stable toxins are elaborated by common molds under uncommon conditions of storage and handling. Such a toxin has been found recently in peanuts; another one was found earlier in certain batches of rice; mycotoxins have been reported in many products. Biological measurements as a means of control are too slow and too costly. With the recent progress in determining the structure of some of the toxins, there is a reasonable possibility that chemical tests can be devised specifically for each toxic material.

The ultimate widest utilization of protein concentrates will depend on their being produced with constancy, that they will be safe, and that there will be reasonable assurance that this safety can be guaranteed. Of course, there must also be improvements in the methods of processing and handling to yield the best products.

b. Position of High Concentrate Flours (70 percent protein). Another barrier to broad usage is the limitation of color and flavor. The amount of soybean protein flour which can be incorporated into bread or pasta is limited by the level which will not interfere with flavor and baking properties. It would be wonderful if cottonseed flour could be incorporated into tortillas, but this would change the color. Peanut products have a characteristic flavor which is generally desirable, but this may interfere with incorporation of these flours into some products. To overcome this limitation it is possible to produce higher concentrates which have less nonprotein material and consequently less distinctive flavor, color, and odor. It is possible, for example, to produce a soybean concentrate containing 70 percent protein which is free of the soybean flavors, and is colorless; it is being incorporated into foods for which the ordinary flour would not be suitable. Likewise, it is possible to extract cottonseed flours with suitable solvents to remove most of the color, and the gossypol. Although such products overcome some disadvantages of the simpler product, they are more expensive. They are more expensive for two reasons: First, there are extra operations, and secondly, and probably a more important reason, the materials removed, principally carbohydrates, are now a waste product; the remaining protein concentrate has to carry the economic loss of the materials that had been removed. Were such byproducts to find their way into higher priced markets, then perhaps the cost of this higher concentrate would be less. Nevertheless, these concentrates because of their greater versatility have a place.

c. Question of Structure. Let us go one step further and inquire whether, if we eliminated the problems of odor and flavor, the presence of deleterious materials, and assured constancy of nutritive value, this would be all that is in the way of unlimited applications of these protein sources in the human dietary?

I believe that the incorporation of flours will be limited primarily to simple, unsophisticated foods. Although there is an extensive literature on the present world protein shortage, I have found very little enthusiasm for the solution to this problem with products not of animal origin. This is so, even though all clearly recognize that there is a limit to the amount of new animal products that can be produced, and that these are intrinsically more expensive. The reason for the reluctance, probably, is that animal products have the additional virtue of aesthetics, of looking and tasting well, of having structure, of being substances around which a meal can be organized instead of being a structureless component of a soup or an "atole". This I believe is the ultimate limitation of the protein concentrate flours, and this is a real limitation. If one were to substitute for all of the presently available animal sources of protein, vegetable sources so designed as to be absolutely equivalent nutritionally, such would still represent a lowering in the stand-

ard of living because it would represent a lowering in the aesthetic quality of the diet.

This is not a new problem. In a sense this is the problem faced by society millenia ago, of devising means of handling the major source of food - the cereals - in an interesting way. Rice, of course, had the advantage that the rice grain is maintained intact during milling. Bread is one of the early successful attempts to create structure out of wheat products and is probably one of the most important inventions of food science of all times. Another invention in the same vein is pasta. Here are at least two ways of handling wheat and wheat products in forms which contain structure and hence interest. These are structured products but these are not protein concentrates.

We might ask whether it is equally possible to produce structured products from protein concentrates; the answer is already available. There are methods for the extrusion into fibrous forms of casein, peanut, soybean, and cottonseed protein; these can be fabricated into structured products. Dr. M. L. Anson has been a pioneer in this effort. He has shown how gel consistency can be produced in products from seed proteins to give them the property of chewiness.

At the last meeting of the Institute of Food Technology in Detroit there were exhibited ham-like, chicken-like, and beef-like products from soybean protein. I felt that this was but a beginning; that one should be not limited to natural forms; and the widest imagination should be applied to the development of appropriate structure forms. Much of the technology required for structure is now available. The question is whether it can be done at low enough cost to be an effective component of protein rich foods.

One might point to another advantage of these structured foods; in the process of manufacture they can be blended with other proteins and other ingredients to improve overall nutritive quality. This would eliminate the necessity for supplementation at other stages and eliminate the problem that often arises of over-supplementation.

A high-protein concentrate is required in order to produce a fibrous character; isolates (95 to 100 percent protein) are generally used. Protein isolates are made by extracting protein from the 50 percent protein concentrates and precipitating the solubilized protein by acid. Isolates can also be made directly from the seed as has been made from peanuts in a modern way by Chayen and associates. There is also the ancient Oriental procedure for isolating proteins from soybeans: Beans are ground in water; the extract is coagulated by heating with sea water to yield a curd. I do not know whether 70 percent concentrates would be suitable for spinning into fibers; this is certainly a possibility that might well be investigated.

The complete protein isolates have the disadvantage of higher cost. Moreover, the protein value of complete isolates is lower. This arises because the proteins are fractionated during isolation. Neither of these need be permanent disadvantages. The cost might be reduced by finding more valuable outlets for the byproducts; the nutritive value improved by blending or supplementation.

d. The Panorama of Oilseed Protein Concentrates. This is the panorama of protein concentrates from oilseeds. One might question my right to include in a discussion of protein concentrates for developing countries such sophisticated products as fibrous proteins, when we will be lucky if we can come to grips successfully at this meeting with even the simplest of the concentrates. I quite agree that one must learn to crawl before he walks; simple things must come first. It would be a great mistake, nevertheless, not to envision the entire picture; even if we are successful with the simple products, this would be a clumsy and limited effort and bound never to provide a permanent or satisfying solution. Even now, there should be some thought and there is a place for the more complex products from seed proteins. It should also be remembered that the Orientals found it necessary thousands of years ago

to go past the simplest products when they chose such advanced products as soybean curd.

Some Final Comments

a. The Need for Sophistication. An important body of opinion holds that we should concentrate on simple approaches to increasing protein supplies--ones which could be adopted at the home and village level--rather than worry about sophisticated approaches which require a highly developed technology backed by science. Obviously there are places where simple methods are appropriate, and perhaps the only approach for some time to come. In general, I would think that the problem of solving protein deficiency is no different in principle than any other problem; solutions are found by increasing sophistication both in the understanding and the approach. It would be futile indeed to thrust upon the housewife the entire burden of improving food handling. There will have to be improved practice of food science in all countries and this will require concomitant development of other industrial and technical capabilities. The fact that this has not come to pass yet in many countries should not relieve us of our responsibilities to advance the technical nature of food science, even as we try to develop simple stop-gap solutions. The ultimate limiting factors are the limitations of science and technology, the need for further information on the commodities on their constituents and their properties, and the development of procedures for making products suitable to the level of requirement. Ultimately, with sufficient advance in our knowledge of science and technology of foodstuffs, there should be no limit to the utilization of products such as those from oilseeds and fish, and by suitable analogy to the products from grass, legumes, microorganisms, or algae. One can conceive a full range of protein concentrates from simple cheap materials to more complex, more expensive materials; all of them, however, intrinsically cheaper than animal proteins.

I have been accused when I talk this way of being "anti-gourmet". I answer by stating that the gourmet is restricted to the natural foodstuffs. Think of what would happen if there were no such restrictions, if there were available as many forms as the mind could envision and as many flavors as the palate could dream up.

b. Fundamental Research on Seed Proteins. It is because of my strong feeling about the ultimate possibilities in oilseed proteins for humans, both in developed and developing countries, that I took the step over 5 years ago of initiating extensive fundamental research on the botany and chemistry of seed proteins. The objective was to determine the uniqueness of the proteins as they exist in seeds and to determine the chemistry of pure seed proteins. This is a story all of its own. I would only want to point out to you that we now know at least one element of uniqueness of seed proteins. All seeds that have been examined contain in their storage tissues bodies which have now been identified as protein bodies. This is true of the cottonseed, of the peanut, of the pea, and the wheat kernel, and of many other seeds. We might even say that the difference between seeds of high and low protein content could reside in their population of protein bodies. Therefore, as we become more acquainted with the proteins in these bodies we begin to arrive at an understanding of the uniqueness of seed proteins and acquire the capability of isolating them better so that we may study them further. Ultimately we would hope that one would not have to deal with seed proteins blindly but with the full knowledge of their physiology and chemistry.

c. Position of Advanced Countries. Finally I would like to retract the position which I have taken for some years, and which perhaps others have taken. It has frequently been stated that it is a great shame that those countries which have the science and technology have an overabundance in animal protein and do not need other forms; but the countries which need the new sources of protein do not have the science and technology. Advancement in the undeveloped countries would therefore depend on the goodwill and kindness of the countries which have the know-

how. This is no longer entirely true; the advanced countries need new forms of protein as well. The point is brought forth full to mind by the popularity of various products for control of obesity. These low calorie products contain large quantities of protein concentrates, principally from milk and soybean. If a person is strongly motivated to control the caloric content of his diet or otherwise control the ingredients, he can do so, but the products available to him now are completely lacking in interest, are formless shapeless powders, or liquid drinks. He must substitute these for the more attractive structured animal foods to which he is accustomed.

As medical and nutritional research develop more information on the relationship between diet and health, there will be more need for controlling the nature of the food intake, for at least a portion of the population. But people will not want to reduce the standard of their diet in terms of aesthetic value, and they need not do so if the structural potentiality inherent in the products that I have mentioned can be developed. We, therefore, can envision a concordance of interest between the countries which have the technology and those which do not, perhaps for different reasons--the one to provide a more versatile food dietary to take care of those who must control the type of food they eat, and the other to provide sufficient cheap protein to eliminate malnutrition

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FIRST PANEL DISCUSSION

Question: What will the relationship of the Southeastern Research Laboratory be to Cotton Research?

L. A. Goldblatt: It is my understanding that all of the research with oilseeds and with peanuts at our Southern Laboratory will be transferred to the new Southeastern Laboratory. This will include transfer of the work with cottonseed.

Question: What is relation between gossypol and cyclopropene acids?

L. A. Goldblatt: I would prefer not to use the word "relation" in connection with these two materials as they are quite different. Gossypol causes yolk discoloration. It is thought cyclopropenoid acids contribute to egg white discoloration. With regard to the chemistry of gossypol and cyclopropenoid fatty acids; they are of completely different chemical structures.

Question: In the new types of cottonseed, are the cyclopropenoids present in the oil?

L. A. Goldblatt: Yes, the oil of glandless varieties of cottonseed contains the cyclopropenoid acids.

Question: Is it found in the new types of meals?

L. A. Goldblatt: Yes, if the meal contains oil.

Question: As I understand, the prepress, solvent meal was of lower gossypol and higher quality when a certain amount of hulls was present in the process as against a more pure meal operation. Why would this be so? Were seed in processing of same gossypol content or of varying quantity? Why would adding hulls in process improve protein quality? I would like further enlightenment!

G. A. Harper: Yes, Bressani reported that the prepress, solvent-extracted meal was of higher quality when more hulls had been added during processing. I consider that Bressani's observation requires confirmation and that any beneficial effects of the hulls must yet be explained. One possible explanation might be that the added hulls cause lowering of gossypol without any lowering of lysine availability. The nutritive results would appear to indicate this had happened. We certainly cannot offer any precise reason. We need more research to learn the answer to your question.

A. C. Wamble: I'd like to ask Vernon Frampton to give us his opinion about this matter. Vernon, perhaps you can tell us in not more than one minute of time.

V. L. Frampton: I think some of the differences may be due to the gossypol method of analysis. Free gossypol determination is an artifact of the method of analysis employed. I don't know the exact answer to your question.

Question: Was the resin used in the cotton batting the phenolic or urea type?

H. L. E. Vix: We tried many types of both urea and phenolic resins. The phenolic types are objectionable as they present an odor-problem. With the other type, we get odor-free products.

PROGRESS IN IMPERMEABLE SEEDCOAT RESEARCH

by
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(Presented by M. N. Christiansen)

Cottonseed quality loss is of serious consequence in the Southeastern and Southern cotton growing areas. For example, in the 4 year period, 1957-60, less than 40 percent of the cottonseed produced in Louisiana was of prime quality; Mississippi, Alabama, and Georgia fared little better. In 1960 alone, the loss from field deterioration was estimated at nearly 11 million dollars. The price discount per ton for excess moisture, or free-fatty-acids (FFA), for the Coastal and Central regions averages $7.73 and $5.45 per ton, respectively, according to a National Cottonseed Products Association report.

The problem is not new, nor has it been neglected by research. Many reports (3, 4) have described the conditions conducive to cottonseed quality loss; namely, sufficiently high moisture conditions for the seed to imbide moisture in excess of 12 to 13 percent, and sufficiently high temperatures for biological activity. Deterioration will not proceed rapidly, however, unless seed moisture is high irregardless of temperature level.

A new research approach on prevention of deterioration was initiated in the late 1950's (1, 2). It might logically be called a "back-to-nature move" because we seek to utilize the devices by which seed of wild cotton species survive from season to season.

An impermeable seedcoat is the primary seed protective device found in wild cottons. It also occasionally occurs in domestic stocks. The impermeable coat actually excludes water once the seed has matured and dried. There are several methods of rendering the seed permeable prior to planting. The simplest is a 2 minute emersion in hot (180°F.) water (5).

The impermeable seed research program initially had three phases aimed at:

(1) determining the usefulness of "hard seed" in preventing seed deterioration,
(2) determining the heritability of "hard seed",
(3) transferring the character to agronomically desirable types of cotton.

The evaluation program consisted of field weathering tests to compare normal and impermeable seed selections. Seed of hard seeded and normal stocks were harvested at monthly intervals during the fall and winter of the 1961-62 and 1962-63 seasons at Stoneville, Mississippi. Seed quality was evaluated by germination tests and by assays of the free fatty acid content of the oil. Tests in 1963-64 were located at Hartsville, South Carolina, Auburn, Alabama, and Baton Rouge, Louisiana.

Seed quality was high in early season harvests in 1961 (Table I). Only a slight loss in germination had occurred by December in normal seed and a considerable loss occurred by January. The impermeable seed quality remained high. The seed quality data from the 1962-63 test reflect the adverse weather conditions prevalent during the boll opening period in September and October. Rotting of bolls contributed much to the low seed quality. This is evident in the differences between entire plant harvests and those in which rotted bolls were excluded (Table I). Even though boll rotting was severe in the impermeable seed selection plots, germination and free-fatty-acid level were still reasonably good in seed from the entire plant harvests. When rotten bolls were eliminated, seed quality was excellent.

TABLE I

Germination and free fatty acid in the extracted oil of cottonseed of permeable and nonpermeable seedcoat selections after field weathering

Harvest date	Germination, %		% free fatty acid in oil	
	M-8 Permeable	16B-7 Impermeable	M-8 Permeable	16B-7 Impermeable
1961-62 Season [1]				
November 10	96	94	0.45	0.17
December 4	89	95	.63	.31
January 2	51	91	2.41	.39
1962-63 Season [1]				
November 15	33	80	10.54	2.03
December 15	22	84	11.57	1.98
January 15	9	74	13.38	2.72
1962-63 Season [2]				
November 15	59	91	4.00	.61
December 15	40	93	5.71	.95
January 15	16	85	10.42	1.25

[1] Entire plant.
[2] Rotted bolls excluded.

The preliminary results from the area-wide test of 1963 were similar to those obtained at Stoneville. Germination and free-fatty-acid data were not yet available at the time of preparing this report; however, tetrazolium quick test data are completed (Table II). The seed from first and second harvests at all locations was of good quality. This, of course, is a consequence of the dry harvest season in 1963. The final harvest in Alabama and South Carolina gave some evidence of quality loss in the permeable seed, while quality remained high in the impermeable seed.

TABLE II

Percentage of high quality and germinatable seed by tetrazolium vigor classification of cottonseed of permeable and nonpermeable selections after field weathering, 1963

Location	Month of Harvest	Permeable		Impermeable	
		Germ.	Vigor	Germ.	Vigor
Auburn, Ala.	October	87	84	100	96
	November	81	78	100	93
	December	78	54	98	92
Hartsville, S. C.	November	87	87	100	100
	December	90	80	94	93
	January	86	52	97	96
Baton Rouge, La.[1/]	October	93	78	97	87
	November	87	87	97	87
	December	90	78	99	98

1/ Some incidence of boll rot.

The present findings of this phase of the hard seed research are most promising. On the basis of these and other storage studies (1, 2), it is concluded that a heritable impermeable seedcoat can materially reduce field and storage deterioration of cottonseed under unfavorable high moisture conditions.

The breeding and genetic portion of our impermeable seed program is in the fourth year of work. The first crosses of an inheritance study were made in 1961 between 16B hardseeded Hopi and 18 upland varieties. The aims of this work were not just to find out how the impermeable seedcoat is inherited, but also to transfer the impermeable seedcoat to a wide range of commercial cottons. This material is currently being screened for a combination of desirable agronomic characters and hard seedness.

Another approach to the problem of getting commercial cottons with an impermeable seedcoat has been to screen commercial varieties. The methods involved are simple but quite effective. Seed of commercial varieties are soaked in water for several days and then frozen at 0°F. for several days. This process of soaking and freezing kills all soft seed while nonimbibed (impermeable) seed survive. Following freezing the seed are hot water treated and planted in the field where the survivors germinate. Impermeable stocks of four upland varieties have been selected in this manner. The progeny of these selections are now being screened for impermeable seed. If this procedure continues to prove successful, we will be a lot closer to good agronomic hard-seeded selections than if we have to depend upon transference of the character from more primitive genetic material.

In summation, an impermeable seedcoat appears to have considerable potential in reducing field and storage deterioration of cottonseed, and the condition is genetically controlled and can be selected from commercial variteties as well as wild cottons.

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STATUS OF BREEDING FOR GLANDLESS COTTONSEED

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It has long been known that substances in the pigment glands of cotton cause undesirable effects when cottonseed is crushed for oil and meal. Gossypol is a main constituent of these pigment glands. It causes discoloration of the oil, lowers the nutritive value of the meal, and is toxic to swine and poultry when fed in excessive amounts. Other substances in the pigments may also contribute to these detrimental effects. In addition to the above undesirable effects, eggs held in storage develop objectionable yolk discoloration when layers are fed meal from glanded cottonseed.

After several years' work at the U. S. Cotton Research Station, Shafter, California, S. C. McMichael by 1956 had successfully produced a glandless cotton strain. This strain was derived from a cross between Acala and Hopi Moencopi, a noncultivated primitive cotton from Arizona. The original glandless strain had very few desirable agronomic features except its glandlessness.

Upon the development of this glandless strain, work was started to transfer this character into commercial types of cotton. At Shafter the glandless genes were introduced into several Acala strains. Under the direction of James R. Meyer of the Delta Branch Experiment Station, Stoneville, Mississippi glandless genes were transferred into several of the rainbelt varieties.

McMichael (1960) reported that glandlessness was simply inherited, being controlled by two recessive genes (gl_2 and gl_3). Because of its simple inheritance the backcross method was used to transfer the glandless character into commercial cotton.

In the backcross method a cross is made between two parents, one called the nonrecurrent parent and the other the recurrent parent. Offspring from this cross are crossed back to the recurrent parent; this is called the first backcross generation. Offspring from the first backcross generation are then crossed back to the recurrent parent to obtain the second backcross generation. This procedure is continued until the breeder reaches his desired goal. The breeder attempts to recover a strain having essentially all the features of the recurrent parent plus the addition of the one or two desirable characters from the nonrecurrent parent. Theoretically, five to seven backcross generations are sufficient to accomplish this. After the last backcross, plants are selfed and desired plants selected out to obtain true breeding lines.

In a glandless breeding backcross program the nonrecurrent parent is the glandless strain and the recurrent parent is a commercial variety, or advanced breeding strain. The goal is to obtain a cotton equal to the recurrent parent in plant type, yield, lint percent, disease resistance, fiber quality, and other features plus the addition of glandlessness from the nonrecurrent parent.

One essential feature in using the backcross technique is to be able to recognize plants carrying the glandless genes. This can easily be done by growing each backcross the second year. Miravalle and Hyer (1962) developed a practical method of identifying plants carrying the glandless genes in the first generation after the backcross. This discovery resulted in cutting

in half the number of years required in the backcross program.

In most cotton breeding endeavors, breeders cross selected parents and then in resulting generations selected simultaneously for several characters, trying to develop a new strain improved in a number of aspects over existing varieties. Because of its nature the backcross method is not suited for such a purpose. Fortunately, there are now available glandless strains sufficiently advanced so that in some breeding programs glandless parents are used in a cross and selection started immediately on a glandless background for new lines.

As previously mentioned, upon the realization that glandless cotton of commercial worth was a possibility, work was started to transfer this character to some of the commercial varieties. At Shafter transfer was started into Acala types and at Stoneville into rainbelt varieties. Because of the many varieties grown from the East Coast through Texas, it was realized that the Delta Station could not undertake to introduce glandlessness into all of these varieties. Therefore ten varieties, representative of the types grown in this vast area, were selected to have the glandless genes transferred into them. The object of the work at Stoneville was to carry these varieties through the fourth backcross and in the process make these stocks available to private and public breeders for sources of the glandless genes.

A winter breeding nursery is maintained at Iguala, Mexico, where private and public breeders from the United States can grow their stocks. It is here that pollen has been made available to all breeders from the glandless lines being developed at Stoneville and Shafter. Many breeders have taken advantage of this offer so that now every important cotton variety is involved in a glandless breeding program. Varieties of Coker, Deltapine, Dixie King, Lankart, Stardel, Stoneville, Wescot, Empire, Rex. Auburn, Gregg, Paymaster, Acala, Pima, and other types have had the glandless genes transferred to them. These varietal types account for over 90 percent of the cotton acreage in the United States.

Recent surveys by Gandy (1962) and C. F. Lewis[1/] made as to the present status of glandless breeding indicate that several breeders have material in the fourth and succeeding backcross generations and now have glandless strains ready for yield tests. Some breeders have already yield tested, but most will be yield testing in 1964 or by 1966. The results of the yield tests already conducted are not available for presentation at this meeting except for the data from Shafter tests, which will be given later in this paper.

Before a variety is released for commercial production, it generally is yield tested over several locations for a number of years. Based on this fact and barring unforeseen difficulties breeders are forecasting that glandless varieties will be available in the late 1960's or early 1970's.

There have been reports that glandless strains are late in maturity, rank in growth, low in lint percent, small bolled, and have other defects. Many of these apparent defects have disappeared with further backcrossing so that now it appears that obvious deleterious associations with glandlessness will not be an insurmountable obstacle in a breeding program.

The question of insect susceptibility has been raised. Dr. C. F. Lewis[2/] recently commented on this question by stating "Reports have been received from entomologists that glandless cotton is more susceptible to insect attack than glanded cotton. The theory is that gossypol or some unidentified substance in the pigment glands imparts

1/ Personal communication.

2/ Paper presented at the Conference on Cottonseed Protein Concentrates, Southern Utilization Research & Development Division, Agricultural Research Service, U.S. Department of Agriculture, New Orleans, Louisiana, January 15-17, 1964.

some degree of insect resistance to glanded cotton to the extent that certain insects prefer glandless to glanded strains. These reports are offset to some extent by experience of breeders who have produced several acres of glandless without unusual insect problems. The whole question of the possible influence of glandless on the insect problem is most complex. Breeders and entomologists are both aware of this problem, and cooperative research in this area is contemplated."

By 1962 the glandless breeding at the Shafter Station had reached the stage when some glandless lines could be yield tested. Glandless lines from two strains were included in the test. These two strains were AxTE 25, an advanced experimental strain, and Acala 4-42-77, a component of the commercial Acala 4-42 variety grown in the San Joaquin Valley of California.

The lines tested are listed in Table I. Grown were an AxTE 25 glanded check and two AxTE 25 glandless lines, 25-188 and 25-189. Also included were an Acala 4-42-77 glanded check and nine Acala 4-42-77 glandless lines (77-191 through 77-199). 25-188 and 25-189 were derived from the F_3 generation after the fifth backcross. 77-191 through 77-197 were F_2 selections from the fourth backcross and 77-198 and 77-199 F_3 selections from the second backcross.

Shown in Table I are lint yield, maturity and plant height of the glandless lines, and their checks. None of the lines yielded significantly less than their checks. Two lines, 77-192 and 77-196, gave a significant yield increase over their check. In general, the glandless lines were later than their checks, although 77-192 and 77-193 were the exception. No significant differences in plant heights were detected by the F test from the analyses of variance. That height differences did not exist was difficult to accept because of observations in the nursery on these same lines; also late maturity generally is associated with increased plant heights. At least height differences were assumed to exist and selection for shorter plants was made in the 1962 nursery.

TABLE I

Lint Yield, Maturity, and Plant Height of the 1962 Glandless Yield Test

Entry	Lint lbs./A	Maturity[1] 1st Pick % of Total	Plant Height Inches
AxTE 25 ck.	995	90	50
25-188	959	80[2]	56
25-189	905	81[2]	52
4-42-77 ck.	1061	92	49
77-191	1042	83[2]	53
77-192	1210[2]	87	57
77-193[2]	1113	90	54
77-194	1093	86[2]	52
77-195	1083	86[2]	57
77-196	1205[2]	87[2]	55
77-197	1045	83[2]	54
77-198	955	77[2]	56
77-199	1134	86[2]	60
L. S. D. 5%	144		N. S.

1/ Retransformed from analysis of arcsine transformed data.
2/ Significantly different from check.

Gin and fiber properties are shown in Table II. For these characters the glandless lines compared favorably with the glanded checks.

Although 77-198 and 77-199 compared well with the 4-42-77 check in the characters reported in Tables I and II, their extreme plant variability, rankness and poor plant type made them undesirable for further breeding endeavors.

The total gossypol content of the entries in the 1962 test was determined. [3] The percent gossypol in the glandless lines ranged from 0.0027 to 0.0049 percent, whereas Acala 4-42-77 had 1.0015 percent and AxTE 25, 1.2830 percent.

3/ Grateful acknowledgement is given to Frank H. Smith, Department of Animal Science, North Carolina State College for the chemical analyses.

TABLE II

Gin Turnout, Upper Half Mean Length (UHM), Micronaire (Mic.), and Yard Strength of 1962 Glandless Yield Test

Entry	Gin Turnout %	UHM	Mic.	Yarn Strength 22s
AxTE 25 ck.	33	1.09	4.00	129
25-188	33	1.13[1/]	4.15	121[1/]
25-189	34	1.09	4.09	129
4-42-77 ck.	36	1.13	4.22	131
77-191	34[1/]	1.10[1/]	4.15	131
77-192	36	1.14	4.00	128
77-193	35	1.13	4.09	132
77-194	35	1.10[1/]	4.35	130
77-195	35	1.13	4.06	125[1/]
77-196	35	1.11[1/]	4.15	131
77-197	34[1/]	1.13	4.37	135
77-198	34[1/]	1.12	4.15	136
77-199	34[1/]	1.12	4.22	130[1/]
L. S. D. 5%	2	.02	N. S.	6

1/ Significantly different from check.

In 1963 another glandless yield test was conducted. The entries for this test are listed in Table III. The 4-42-77 glandless lines (77-58 through 77-62) originated from F_3 plant selections made from the fourth backcross. Line 77-58 was derived from a plant selection made in a progeny row whose parent gave rise to 77-194 of the 1962 test. In like manner, 77-59 and 77-60 came from progeny rows whose parents gave rise to 77-195 and 77-196, respectively. In other words, 77-58, 77-59, and 77-60 could be considered as reselected 77-194, 77-195, and 77-196 lines.

AxTE 1-62 is an advanced experimental strain developed at the Shafter Station. AxTE 1 glandless line, 1 gram is a composite of several plants selected in the F_3 generation of the second backcross. This line is not pure glandless. The glandless genes in 1 gram came from the fourth backcross generation of AxTE 25. Whereas the glandless genes for the 4-4277 glandless lines came from one of McMichaels original glandless lines.

Lint yield, maturity, and plant height for the 1963 test are shown in Table III. Lint percent, upper half mean (UHM), micronaire, and yarn strength are given in Table IV. At this time data in Table IV are available from only one replication, therefore the data were not analyzed statistically.

TABLE III

Lint Yield, Maturity, and Plant Height of 1963 Glandless Yield Test

Entry	Lint lbs./acre	Maturity 1st Pick % of Total	Plant Height Inches
4-42-77 ck.	1199	73	50
77-58	1191	74	49
77-59	1091	74	56
77-60	1131	77	51
77-62	1092	71	53[1/]
AxTE 1-62	1246	86	41
1-gs.	1173	81	45[1/]
L. S. D. 5%	123	N. S.	4

1/ Significantly different from check.

TABLE IV

Lint Percent, Upper Half Mean Length (UHM), Micronaire (Mic.) and Yarn Strength of 1963 Glandless Yield Test[1/]

Entry	Lint %	UHM	Mic.	Yarn Strength 22s
4-42-77 ck.	36	1.19	4.05	144
77-58	35	1.15	4.63	144
77-59	36	1.17	4.25	144
77-60	35	1.13	4.20	147
77-62	36	1.19	4.08	154
AxTE 1-62	34	1.17	4.53	138
1-gs.	33	1.18	4.45	146

1/ Data from only one replication.

Data in the two tables indicate the glandless lines compared very favorably with their checks. Of special interest is the fact that the 4-42-77 glandless lines did not differ significantly in maturity from the check and only one line, 77-59, was significantly taller. Evidently selection pressure exerted in 1962 was effective in giving earlier maturing and shorter glandless lines.

The Verticillium wilt tolerance of the glandless lines was checked against that of their checks on land heavily infested with the wilt organism. The wilt tolerance of the glandless lines was equal to that of their checks.

The results of the 1962 and 1963 tests indicate that commercially acceptable glandless strains can be obtained from the fifth to seventh backcross generations.

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OBSERVATIONS ON FEEDING COTTONSEED AND COTTONSEED PRODUCTS

by
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Studies in the Pharmacology Laboratory on cottonseed and cottonseed products have been concerned with the effects on growth of rats produced by various samples of cottonseed meal, pigment glands, gossypol, and the cyclopropene acids of cottonseed oil. They have been concerned with a search for metabolic end products of gossypol, with tests for estrogen-like activity in cottonseed oil and soap stocks, and with tests for aflatoxin in moldy cottonseed and meal made therefrom.

Since many of these studies have been concerned with some manifestation of toxicity it is essential to emphasize the difference between acute and chronic toxicity. Acute toxicity concerns the undesirable physiological effects produced by a single dose. Chronic toxicity concerns the effects resulting from repeated exposure to small doses over long periods of time. It should be emphasized that doses too small to produce any demonstrable acute effects may upon repeated administration over long periods result in toxicity. It should also be emphasized that the symptoms of acute toxicity and mechanism of their production are frequently quite different than those of chronic toxicity. Three examples will illustrate the point. People have been severely poisoned by sodium fluoride used by mistake in lieu of baking powder. The resulting gastrointestinal irritation and the drop in blood calcium level resulted in some deaths. In contrast to this acute toxic reaction, people born and raised in areas where the drinking water supplies contain fluoride far in excess of the amount recommended for reducing the incidence of dental caries develop mottled enamel of the teeth. This form of chronic fluorine toxicity can be produced in rats without evidence of gastrointestinal irritation or drop in blood calcium level. There is, however, a marked inhibition of the enzyme bone phosphatase. A number of years ago we had to determine the acute and chronic toxicity of aminofluorene, an organic compound having potential value as a pesticide. We were unable to give experimental animals single doses large enough to produce acute toxicity. In contrast feeding rats a diet containing as little as 40 parts per million, i.e. 0.004% produced a wide variety of cancer. Of more immediate interest to everyone concerned with the storage of a food or feed susceptible to mold growth is aflatoxin, the name given a mixture of organic compounds produced by certain strains of the mold Aspergillus flavus. A few micrograms will kill a duckling, but I am told that the daily ingestion of an amount too small to have an acute effect will result in liver cancer.

The examples cited emphasize two points which differentiate acute and chronic toxicity. The daily dose required to produce chronic toxicity is small compared with the single dose responsible for an acute effect. The symptoms and mechanism of their production may be quite different for acute versus chronic toxicity. The points I have tried to emphasize are pertinent to the problems of toxicity of pigment glands and gossypol.

Our first feeding experiment on rats involved the effects of two samples of gland-free cottonseed meal, two pigment gland samples, and a sample of hexane-extracted meal. The gossypol content of these samples is shown in Table I.

TABLE I

Gossypol content of samples

Sample	% Gossypol
Gland-free meal #1	0.057
Gland-free meal #2	0.076
Pigment glands #1607-E-11	37.5
Pigment glands #1635-E-5	36.5
Hexane-extracted meal	1.1

Meal Studies

Feeding gland-free sample #1 to male rats for 185 days at dietary levels of 8, 16, and 32% gave no evidence of toxicity as judged by growth, hematological examination, and histopathological examination of thyroid, lung, heart, adrenals, kidney, liver, spleen, stomach, intestine, pancreas, and testes. The same was true of male rats fed dietary levels of 7.5, 15, and 30% of gland-free sample #2 for 195 days.

However, the hexane-extracted meal containing 1.1% gossypol fed at a dietary level of 15% (equivalent to approximately 0.16% gossypol) for 195 days caused a growth inhibition not accounted for by a decreased food intake, Three of the five rats died on the 21st, 28th, and 56th day. When the dietary level of the hexane-extracted meal was increased to 30% all rats died within 21 days. This level of hexane-extracted meal was then fed to 5 more mature rats having an average weight of 104 grams. These rats lost weight. One died on the 13th day, 2 on the 20th, and 2 more on the 27th. These data are summarized in Tables II and III.

Pigment Gland Studies

Male rats fed a diet containing 0.256% pigment gland sample #1607-E-11 (37.5% gossypol) for 185 days showed growth inhibition (140-gram gain compared with 257 for controls). In contrast, male rats fed the same dietary level of pigment gland sample #1635-E-5 (36.5% gossypol) for 195 days

TABLE II

Hexane-extracted cottonseed meal (1.1% gossypol) in diet of male rats (5 per group)

Meal in diet, %	Weight per rat, grams: At start	After 195 days	Time of death, days
None	42	300	195 (autopsy)
3.75	42	306	195 "
7.5	42	291	195 "
15.0	43	180, 232(2 rats)	21, 28, 56
30.0	42	---	21 (all dead)

TABLE III

Hexane-extracted cottonseed meal (1.1% gossypol) in diet of male rats (5 per group)

Meal in diet, %	Weight per rat, grams: At start	6th day	13th day	Time of death, days
30	104	86	84	13 (1 rat) 20 (2 rats) 27 (2 rats)

grew normally, but growth inhibition and some deaths occurred on a dietary level of 0.512%.

In a separate feeding trial with pigment gland sample #1607-E-11 fed to both male and female rats at a dietary level of 0.256% for 100 days, reproduction failed to take place. When female rats on the same diet were mated with male rats kept on the control basal diet up to time of mating all cast litters, but only one litter survived. The females were repeatedly remated with males on the 0.256% pigment gland diet without success. These results suggest that the lack of fertility is in the male rats fed pigment glands.

Paired feeding experiments using both male and female rats were performed on 0.192% gossypol in the daily diet, and on 0.256% and 0.512% pigment gland sample #1607-E-11 (37.5% gossypol). The results clearly showed that growth inhibition on the

gossypol and pigment gland containing diets was greater than could be accounted for by the reduced food intake.

Acute Toxicity

Following the above feeding tests, limited information on the acute toxicity of gossypol and pigment glands was obtained. Fifteen rats were given oral doses of gossypol ranging from 200 to 800 milligrams per kilo of body weight with only one death on a dosage of 400 milligrams. Two rats given 1,600 milligrams and two given 3,200 milligrams died in 6 to 9 days. Forty rats were used in evaluating the toxicity of a pigment gland sample containing 25.9% gossypol and 2.59% gossypurin. Oral doses ranging from 2,000 to 4,000 milligrams per kilo of bodyweight were given in increments of 500 milligrams. No deaths occurred on doses of 3,000 milligrams or less. Three of 7 rats given 3,500 milligrams, and 6 of 7 rats given 4,000 milligrams died. Another sample of pigment glands containing 27.6% gossypol and 2.5% gossypurin proved to be more toxic in a series of 17 rats. No deaths occurred on doses of 500 and 1000 milligrams per kilo of body weight. One of three rats died on 1,500 milligrams. All of the 8 rats distributed evenly on doses of 2,000, 2,500, 3,000 and 3,500 milligrams died. Table IV summarizes these data.

The following Table V is reproduced from the Handbook of Toxicology issued by the Division of Biology and Agriculture of The National Academy of Sciences gives information which would classify gossypol and both pigment gland samples as slightly toxic. This, of course, refers to acute toxicity and would not enable one to predict the toxicity observed in feeding test with the hexane-extracted meal or a basal diet to which 0.256% pigment glands had been added.

TABLE IV

Pigment gland sample				Gossypol Sample	
Gossypol, 25.9% Gossypurin, 2.6%		Gossypol, 27.6% Gossypurin, 2.5%			
Amount fed, mg./kg.	Deaths/ Total	Amount fed, mg./kg.	Deaths/ Total	Amount fed, mg./kg.	Deaths/ Total
				200	0/2
				400	1/2
		500	0/3	500	0/3, 0/3, 0/3
				800	0/2
		1,000	0/3		
		1,500	1/3		
				1,600	2/2[1/]
2000	0/7, 0/7	2,000	2/2		
2500	0/5	2,500	2/2		
3000	0/7	3,000	2/2		
				3,200	2/2[1/]
3500	3/7	3,500	2/2		
4000	6/7				

1/ Delayed deaths, 6 to 9 days.

TABLE V

Toxicity rating	Commonly used terms	LD_{50} Single oral dose, rats
1	Extremely toxic	1 mg. or less/kg.
2	Highly toxic	1-50 mg.
3	Moderately toxic	50-500 mg.
4	Slightly toxic	0.5-5 g.
5	Practically non-toxic	5-15 g.
6	Relatively harmless	15 g. and more

In view of the demonstrated toxicity of pigment glands and gossypol to rats in terms of growth inhibition, longevity, and reproduction, we were completely surprised by the following observation for which we have no explanation at the moment. A sample of cottonseed meal, known to have killed 85% of a hog population, was fed to rats and compared with a high quality cottonseed. Growth and reproduction were good on both cottonseed meal samples and compared favorably with soybean meal.

Miscellaneous Tests

More than 30 years ago Dr. E. P. Clark found that the LD_{50} dose of gossypol in cottonseed oil injected intraperitoneally in rats was 20 mg. per kilo of body weight, and that the corresponding dose of apogossypol was approximately 80 mg. Since there are no aldehyde groups in apogossypol, it might be concluded that the toxicity of this compound involves a mechanism other than combination with lysine. We have repeated Dr. Clark's work using mice instead of rats and confirmed his results.

Because of our extensive experience in working out the metabolic fate of numerous compounds having phenolic hydroxyl groups, and because of the number of such groups in the gossypol molecule, a study of its potential metabolic products seemed worthwhile. It was hoped that information obtained might throw light on the mechanism of toxicity and suggest means of detoxification.

Gossypol was given to rats by stomach tube and the urine examined for phenolic degradation products. The results were negative, suggesting a lack of absorption from the gastrointestinal tract. Other rats were, therefore, given gossypol by intraperitoneal injection. A dosage of 100 mg. per adult rat, suspended in isotonic saline proved fatal, but survival occurred when 60 mg. of lysine were injected at the same time. However, no evidence of phenolic metabolites appeared in the urine. It is tempting to speculate that the failure to metabolize gossypol and the toxicity may be different manifestations of the ability of gossypol to inactivate enzymes by combining with lysine or other amino acids of the enzymes. At the March 19-20, 1959, Conference on The Chemical Structure and Reactions of Gossypol and Nongossypol Pigments of Cottonseed, T. M. Ferguson, J. R. Couch, and R. H. Rigdon reported as follows: "Biochemical studies indicate that oxygen uptake and xanthine oxidase activities are reduced following in vitro addition of gossypol acetate to chick embryo homogenates. Gossypol acetate also inhibited succinic dehydrogenase and cytochrome oxidase activity."

M. S. Masri, A. N. Booth, and F. DeEds have reported on the O-methylation in vitro of dihydroxy- and trihydroxyphenolic compounds by liver slices (Biochem. Biophys. Acta, 65: 495-505, 1962) and demonstrated that protocatechuic acid (3, 4-dihydroxybenzoic acid) is methylated to form vanillic acid (3-methoxy-4-hydroxybenzoic acid). In a preliminary experiment it has been shown that gossypol prevents methylation of protocatechuic acid by liver slices. In another preliminary experiment the effect of gossypol upon the ability of the rat to metabolize caffeic acid was studied. Each of 2 rats was fed an adequate basal diet containing 1.0% caffeic acid. To the diet of one rat 0.15% gossypol was added. The amount of food consumed each day by this experimental rat determined the amount of gossypol-free diet weighed out for the control rat, thus assuring approximately the same intake of caffeic acid by both rats. At the end of 1

week a 24-hour sample of urine was collected from each rat. Each urine sample was acidified with hydrochloric acid and extracted with ether. Aliquots of the ether extracts were subjected to two-dimensional chromatography for identification of metabolites by the procedure described by A. N. Booth, C. W. Murray, F. T. Jones, and F. DeEds (J. Biol. Chem., 223: 251, 1956). The metabolic transformations of caffeic acid shown in Figure 1 involve dehydroxylation of the benzene ring, methylation of a phenolic hydroxyl group, hydrogenation, and dehydrogenation of the side chain, shortening of the side chain by β-oxidation, and conjugation of a phenolic hydroxyl group with glycine or glucuronic acid.

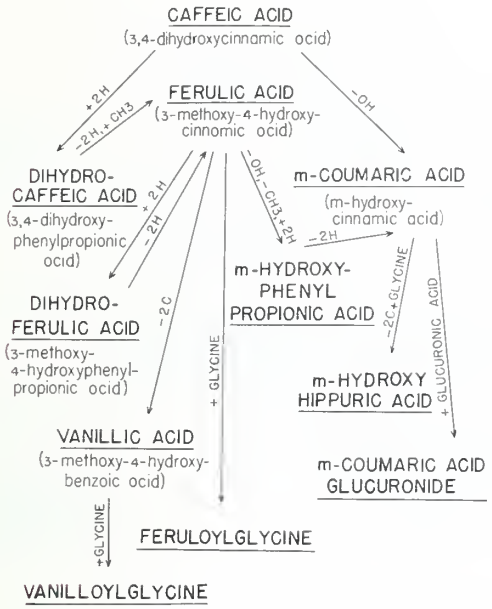

Figure 1

Proposed scheme for the metabolic transformations of caffeic acid.

Comparison of the 2 chromatograms suggested that the rat ingesting gossypol excreted decreased amounts of the meta hydroxy derivatives of phenylpropionic, coumaric, and benzoic acids indicative of decreased dehydroxylation of the benzene ring by intestinal microorganisms (A. N. Booth and R. T. Williams, Nature 198: 684, 1963). A decrease in β-oxidation of the side chain was suggested by decreased excretion of vanillic acid and its glycine conjugate. The decreased excretion of vanillic and ferulic acid and their glycine conjugates also suggested that O-methylation may be inhibited in vivo.

The fact that ruminants are more tolerant of gossypol than is the case with non-ruminants suggests the degradation of gossypol by rumen microorganisms. Such organisms were not available but culture media containing a 0.4% suspension of gossypol were innoculated with Penicillium expansum, Rhizopus nigricans, Alternaria, and Polyporus circinatus and incubated for 6 days. All organisms were able to grow and degrade the gossypol as evidenced by clearing of the turbid culture media, and by study of absorption spectra at different time intervals.

There is an appreciable lack of agreement among various investigators as to the level of gossypol that can be tolerated by various animal species. Undoubtedly many variables are involved, one of which may be the influence of naturally occurring oxidants and antioxidants in the basal diet, especially when ordinary rather than purified diets are used. Evidence in support of the antioxidant concept was reported by C. A. Cabell and I. P. Earle (J. Animal Science 20: 390, May 1961) on the "Effect of Synthetic Antioxidants and Flavonoids Present in Cottonseed on Gossypol Tolerance in the Rat." We performed a similar experiment using ferric chloride as an oxidant. In confirmation of Cabell and Earle, the toxicity of gossypol was increased by adding quercetin to the diet. When ferric chloride was added to the gossypol-containing diet, toxicity was reduced. Table VI summarizes the results of the experiment in terms of weight gain, food intake, and food efficiency.

In view of these results a second experiment in which the dietary level of gossypol was raised from 0.1 to 0.15% was run. The following oxidants and antioxidants were then fed to groups of 3 rats as individual additives

TABLE VI

Modification of gossypol toxicity

Diet	Avg. wt. gain [1] (gms)	Food intake g./rat/day (gms)	Food efficiency [2]
1. Control	98	10.5	0.33
2. " + 0.1% quercetin + 0.1% $FeCl_3$	100	10.6	0.33
3. " + 0.1% gossypol	59	99.0	0.24
4. " + 0.1% gossypol + 0.1% quercetin	48	8.1	0.21
5. " + 0.1% gossypol + 0.1% $FeCl_3$	87	10.5	0.30

1/ 3 rats per group

2/ Food efficiency = gain in weight ÷ total food consumed

(0.15%) to the gossypol-containing diets: quercetin, dihydroquercetin, ascorbic acid, ethoxyquin, sodium bisulfite, ferric chloride, and ferrous chloride. As judged by weight gain in a 28-day period, quercetin and ascorbic acid enhanced the toxicity of gossypol, whereas both ferric chloride and ferrous chloride decreased the gossypol toxicity. The other compounds had no effect.

Estrogen-Like Activity of Cottonseed Oil and Soap Stocks

During the course of mouse assays for estrogen activity, it was discovered that corn oil was slightly active. This was followed by assays on a number of oils and the results were published in Science, 131: 1807, June 17, 1960. The sample of cottonseed oil tested was refined and obtained from a retail store. Because of its low estrogenic value, samples of cottonseed oil produced by screw-press, hydraulic-press, and solvent extraction, and four acidulated soap stocks were obtained from the Southern Regional Research Laboratory. These samples were assayed for extrogen activity and compared with a sample of Wesson oil. The increases in uterine weights of immature mice, indicative of estrogenic activity, are shown in Table VII.

TABLE VII

Sample	Uterine weight (mg)
Control	11.3
Wesson oil 1.4 g./mouse in diet	17.0
Control	10.4
Wesson oil 1 g./mouse in diet	15.9
CSO-hydraulic 1 g./mouse in diet	16.1
CSO-screw press 1 g./mouse in diet	16.5
CSO-solvent extracted 1 g./mouse in diet	15.4
Soap stocks	negligible

Inhibitors of Biological Process

A number of years ago Dr. A. M. Altschul and associates of the Southern Regional Research Laboratory, conducted studies on the chemical treatment of cottonseed to minimize heating and formation of free-fatty acids in moist cottonseed. As a result of these tests the Pharmacology Laboratory made toxicological studies on Vinyl propionate, ethylene chlorohydrin, propylene glycol dipropionate, and 1, 3-dimethyl-4, 6-bis (chloromethyl) benzene. The results of

these studies were published in 1950 and 1951 in volumes 2 and 3 of the Archives of Industrial Hygiene and Occupational Medicine. Attention is called to the existence of this information because the present interest in the problems created by molds growing on cottonseed and other commodities may cause renewed interest in these compounds.

THE NEED TO STUDY MOLDS ASSOCIATED WITH COTTONSEED

by
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Recently, a cottonseed miller mentioned to me that his seed was coming in with 25 percent moisture. He aerates to cool the seed, but can't dry it rapidly. What is he worried about? Suppose he does have wet seed? Well, he knows that fungi or molds can grow in high moisture seed. And molds produce heat within a seed pile--enough heat to burn up the seed and the storage building with it, if it is not cooled in some way. The heat in turn changes the color of the oil within the seed. Molds produce even more moisture during their growth, but worse than this--a number of molds can break down the oil within the seeds and cause acidity in the oil. All of this activity reduces the quality and value of the cottonseed and the yields of high quality oil derived from it.

Since the subject has not been discussed in recent processing clinics, it might be useful to review this very important problem in the storage of cottonseed. I would like to show you first, an example of the production of heat in cottonseed by the growth of molds; second, the production of free fatty acids in the oil of stored cottonseed by molds; third, the differences in the actions of various molds on cottonseed oil; fourth, to mention a new problem also caused by molds to be faced in the storage of cottonseed; fifth, to show you a few pictures of the molds that cause trouble; and last, to talk about what can be done to wet seed to protect it.

Dr. Clyde M. Christensen of the University of Minnesota demonstrated very graphically the production of heat by molds (1). He stored seed in an adiabatic respirometer. This is a device which allows no heat to escape or be added, and yet provides adequate ventilation. There were two sets of the same batch of cottonseed at high moisture content (18 percent). One set was untreated and the other was treated with a chemical which Dr. Christensen knew would prevent the growth of molds for a period of time.

In Table I it may be seen that in the untreated seed, which was obviously moldy at 7 days, the mold count was high and the temperature was very much above room temperature. The molds continued to live and to produce heat until finally so much heat accumulated that they were killed by their own respiration. On the other hand, in the treated cottonseed at 7 and 9 days, the seeds were clean and the temperature did not rise. Counts of the molds were not made. By the 16th day, however, the seeds were moldy, both visually and by count, and the temperature rose. Further incubation repeated the trends of growth and death of molds and the rise in temperature which occurred in the untreated seeds.

The production of fat acidity by molds in stored cottonseed is demonstrated in the work done by a former National Cotton Products Association fellow, Mrs. Edith Jensen Conkerton and her coworkers here at the Southern Utilization Research and Development Division (2). High moisture content cottonseed (16 percent) was stored at a high storage temperature (100° F.) and constant relative humidity (92 percent). At intervals, samples of the cottonseed were removed for chemical and microbiological analyses. There were three sets of seed: untreated seed; seed treated with a chemical which prevented the growth of molds for a short time and then lost its activity; and seed treated with chemicals which prevented the

growth of mold for the duration of the storage. When mold growth occurred there was a very large increase in free fatty acids in the oil of the seed.

TABLE I[1/]

Storage of Cottonseed in Adiabatic Respirometer

Days stored	Molds 1,000/gm.	Temp. °C.
	Untreated seed	
7	470	37
9	287	46
16	0	66
	Treated seed	
7	---	26
9	---	27
16	450	33
20	300	47
23	0	57

1/ Data from Christensen, C. M., Jon H. Olafson, and W. F. Geddes. 1949. Cereal Chem. 26: 109-128

These two sets of workers showed that molds produce heat in the seed pile and fatty acids in the oil of the cottonseed. But microorganisms can utilize oil in various ways. Other work at the Southern Utilization Research and Development Division (3, 4) demonstrates the differences in activities of these agents. Microorganisms had been collected from recently harvested cottonseed from many different geographical areas and also from seed that had been stored under various conditions, giving a good sample of the kind of organisms to be found in the seed. When tested, the microorganisms that could break down fat, both bacteria and molds, fell into five different groups according to how much they consumed neutral fat and to the amount of free fatty acids which accumulated in the culture medium containing cottonseed oil. Figure 1 shows the different activities. Group I, all of which were bacteria called Xanthomonas-like, consumed most of the neutral fat and accumulated large amounts of fatty acids. Group II, one of which was a Penicillium sp., used the neutral fat and accumulated only moderate amounts of fatty acids. Apparently they used some of the fatty acids also. The third group, one of which was an Aspergillus flavus, used neutral fats but did not accumulate many fatty acids, and the fourth and fifth were not as actively lipolytic. The first and last groups were bacteria and probably would not grow until the moisture is extremely high. But of the molds, it was noted that it was the so-called storage molds, e.g., the Penicillia and the Aspergilli, rather than the mold plant pathogens which were most active in producing free fatty acids.

At the beginning of this talk, it was mentioned that there is a new problem of which we have been made aware in the last few years. There have been sporadic illnesses occurring in animals which have eaten moldy feed, not necessarily cottonseed. These illnesses have been caused by harmful chemicals produced by the metabolic processes of various molds growing on moist feed which is then ingested. Corn, sweet clover hay, various grains, and processed feeds have been associated with these illnesses. Also, in the past 3 years there have been many investigations on the so-called "Turkey-X" disease. This was an illness of turkeys which occurred in 1960 in the United Kingdom causing the loss of some 100,000 young turkeys in the course of a few months. It was found that a mold called Aspergillus flavus had grown in the feed (which happened to be peanut meal) and had produced a strong toxin which caused the trouble. Not all strains of the mold produced toxin, but some did. Recalling the slide which was shown on the action of lipolytic molds, one of the highly lipolytic molds was Aspergillus flavus, and it was found very often in mold cottonseed.

It is good to be aware of the possibility that this organism is capable not only of causing deterioration of the oil, but also of producing toxin which could stay in the cottonseed meal after processing.

It might be of interest to see the molds that have been discussed here. (Here were shown slides of Aspergillus flavus, A. terreus, A. awamori, Fusarium sp., and Penicillium sp.)

What can be done to prevent the growth of molds? Ideally, the moisture content of the stored seed and also the temperature of storage should be kept low. If the temperature is low, more moisture can be allowed; if the moisture is low, a slightly higher temperature can be allowed. According to Simpson in 1935 (5), 10 percent moisture content or below can be considered safe for cottonseed. Actually it is dependent on the equilibrium set up between the relative humidity of the immediate vicinity of the individual cottonseed deep within the pile and the moisture of the seed. Some molds can grow at 65 percent relative humidity, while others grow best at 85 to 90 percent relative humidity. Karon and Adams (6) found that at 64.4 percent relative humidity, there was set up an equilibrium of 9.6 percent moisture content in whole, shelled peanuts, and at 75.4 percent relative humidity, 11.4 percent moisture; this latter amount is one at which most molds can grow. Various people have done work on drying the cottonseed. Shaw and Franks (7) have published on cottonseed drying and storage right at the cotton gins; and there have been some studies on aeration of seed piles at College Station, Texas. The work on the storage of seed cotton by the investigators at Stoneville might find some application in this area also (8).

The best chemical treatment found at the Southern Utilization Research and Development Division was propylene glycol dipropionate mixed with 1,3,-dimethyl-4,6-bischloromethylbenzene at the rate of 30 pounds per ton of seed. It prevented heating in cottonseed for 73 days (2). There was some preliminary work with maleic hydrazide applied to cotton plants (10). It suppressed free fatty acid formation in the field, inhibited free fatty acids formation in storage for 16, 20, 28 weeks at 100 percent, 91 percent, and 75 percent relative humidities.

To summarize, molds can cause a lot of trouble during the storage of cottonseed. They lower the quality of the oil by producing heat, fat acidity, and other changes in the oil. Some molds are capable of producing toxins during their growth, toxins which can cause illness or death in animals using the meal as feed. And although we know something about the cause of these trouble, we still don't have an exact answer for our friend with his wet cottonseed.

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SECOND PANEL DISCUSSION

Question: Will you comment on insect and disease susceptibility of the hard coat cottonseed?

M. N. Christiansen: It is difficult to comment on something about which we have only the results of the past year's field trials. We expect to have two field trials' results when we have completed this year's work. So far we have not observed any differences in insect and disease susceptibility between the two types of seed.

Question: Why was the work on maleic hydrazide stopped?

Ruth Mayne: I don't know the reason for stopping the work.

Floyd DeEds: I do not know the reason in the minds of those who stopped its use. However, maleic hydrazide has been reported to be carcinogenic, and this could be sufficient reason for stopping the use of it for seed treatment.

Question: What degree of drying is necessary to permit hard seedcoat to function?

M. N. Christiansen: Seed of approximately 12 percent moisture become impermeable.

Question: What makes cottonseed have fuzz on it?

M. N. Christiansen: That is the way most domestic cottonseed grows. Some wild and domestic biotypes produce cottonseed which do not have any fuzz.

Question: Have you done any feeding studies with glandless cottonseed? What were the results?

Floyd DeEds: No, we haven't. I think Dr. Phelps can call on those who have conducted feeding studies.

R. A. Phelps: Does anybody wish to comment on this question? How about you, Garlon?

G. A. Harper: I think I commented as much as you would want at this time. Dr. Watts has fed glandless cottonseed meal and found it to be of high nutritive quality. Under the conditions of Dr. Watt's tests it is equal in quality to high quality soybean meal and vastly better than some glanded cottonseed meals. Those results were obtained by feeding broilers. In the laying hen, both gossypol and the Cyclopropenoid fatty acid (CPA) of the lipid fraction of cottonseed meal will affect egg discoloration. The level of color in eggs is dependent to a large degree upon the CPA of the meal or other fraction(s) occurring with CPA in residual cottonseed lipids. The preliminary feeding studies with swine show the glandless cottonseed meals are very good nutritionally.

Question: Do the molds penetrate the seed coat or do they remain in the lint until the seed are hulled?

Ruth Mayne: Yes, they can penetrate into the seed. You can find a high percentage of internally infected seed in deteriorated seed.

Question: What was the cause of death in animals given; pigment glands by stomach tube; gossypol by stomach tube; and gossypol intraperitoneally?

Floyd DeEds: In acute dosing by stomach tube in rats there is widespread gastrointestinal irritation. This effect is not seen in chronic toxicity due to small amounts fed daily over a period of time. We have evidence suggesting that inhibitory effects on various enzymes may be a factor in chronic toxicity, but have no evidence that such a mechanism plays a role in acute toxicity.

Question: What effect on processing and processing equipment can be expected with impermeable seedcoat cottonseed? Have any studies been made concerning this?

M. N. Christiansen: We have studied the effect of ginning on breakage of the hard seedcoat. Some differences have been observed between saw and roller gins. We have not yet investigated various types of equipment used to process the impermeable seed. In conjecture, when you ask about the effects of hard coat seed versus normal seed, you should not be thinking that hard coat seed resembles rocks or anything like that. The hard seed is very much like the normal seed, except that the seedcoat of the former is less permeable to water due to a seal of the portal of water entry.

Question: Did you autopsy rats after death from pigment glands?

Floyd DeEds: Rats dying acutely after oral administration of pigment glands showed gastrointestinal irritation as evidenced by hyperemia (dilation and engorgement of the blood capillary network).

Question: Do the glandless cottonseed strains under development have impermeable seedcoats?

M. N. Christiansen: The material we used originally for getting our first source of impermeable seedcoat was from a mixture of glanded and glandless cottonseed. It was not a homogenous source to begin with. We have been studying the impermeable seedcoat in seeds obtained from that original source. We have not set up any studies to deal strictly with glandless, impermeable seed.

Question: Are hard seed the dormant seed talked about in the literature of 15 years ago?

M. N. Christiansen: An impermeable seed is a type of dormant seed; however I would not think this is precisely true.

Question: Do you think that storage of damp cottonseed under a carbon dioxide (CO_2) atmosphere will prevent mold growth?

Ruth Mayne: I do not have any information about storage of cottonseed under CO_2 atmosphere. I think some work has been done with grain storage. The theory was given that toxicity of the CO_2 gas and not the lack of oxygen was responsible for inhibiting mold growth.

Question: With regard to the chemical used for treating, does it make the seed unfit for crushing?

Ruth Mayne: No; not the chemical mixture containing propylene glycol dipropionate which was used.

Question: Which one of the following do you think is most harmful to the hen and to the rat: (1) gossypol; and (2) cyclopropenoid fatty acid?

Floyd DeEds: Our studies to date show that gossypol is more harmful to the rat than CPA. We have not worked with hens. The sum total of the work of others shows that the egg must be considered as well as the hen, and when this is done both gossypol and CPA are detrimental. In the case of the rat more work should be done on the effects of CPA on fertility and on the ratio of saturated to unsaturated fatty acids.

Question: What is the theory behind the hard seedcoat?

M. N. Christiansen: There are three types of seedcoat conditions which have to be considered. One type is of seeds with a completely permeable seedcoat that allows free passage of water; the second is of seeds

which mature under dry conditions which result in development of some seeds with the hard coat; and the third is a pure genetic hard coated seed. Hot water treatment and observance of its effect on the chalazal aperture of the seedcoat will distinguish between the hard coated seed and the permeable coated seed. The hard coat seed is truly different genetically from the normally permeable or the environmentally induced hard seed.

Question: Would impermeable seed be affected by molds, etc. ?

Ruth Mayne: I don't know as I have not worked with the seed having the impermeable seedcoats. I think this would depend upon whether the molds could get through the chalazal opening and attack the inner parts of the seed.

Question: Under storage conditions of high humidity, would fungi be present at the chalazal openings?

M. N. Christiansen: Yes, and I think the fungi could penetrate into the impermeable seedcoat.

Question: If gossypol is so toxic, why do the mice and rats around an oil mill grow and thrive so well?

Floyd DeEds: In my discussion, I emphasized the difference between acute and chronic toxicity and showed data indicative of the relatively low acute toxicity of gossypol. I believe it much more likely that mice and rats around an oil mill would die of chronic toxicity rather than acute toxicity. Animals dying acutely would more likely be seen than the chronically ill who go elsewhere and die.

Question: What effect do molds have with respect to the cellulose of the linters?

Ruth Mayne: The molds can break down the cellulose of the fibers.

Question: How can you work 7 percent moisture seed and get results?

Charles Hay: We have seen 7 percent moisture seed worked without any rolling and without any moisture being added to the cooker, and the results were highly satisfactory in this expeller mill.

We are getting high quality products from those seed in the prepress, solvent operations. It is necessary to bring the moisture up to 10 to 12 percent either ahead of rolling or in the cooker.

Question: What percent water in cottonseed meal is safe, that is, not conducive to mold growth?

Ruth Mayne: For whole cottonseed, a moisture content below 9 percent is preferable. For cottonseed meals, this would vary with processing conditions and type of seed. I have not seen data on hygroscopic equilibrium of cottonseed meals in the literature. A safe moisture content would be that which is in equilibrium with less than 65 percent relative humidity.

Question: When glandless seed are planted in the rain grown cotton belt, will it be necessary for all farmers to plant the glandless varieties?

A. H. Hyer: All farmers will not necessarily need to grow glandless varieties, however, some system will have to be worked out to keep glandless and glanded seed separate at the gins and crushing mills. It may be that a gin or even a mill handle only glandless seed in order for the farmer to realize an economic gain from growing a glandless variety. This will depend upon the price of glandless seed. Very likely cooperation among farmers, ginners, and mill men will be necessary to make the growing of glandless varieties in the rainbelt a reality.

Question: Has the boll weevil attractant in cottonseed hulls been identified?

B. H. Wojcik: The attractant for the female weevil has been isolated from the male.

Question: Is it practical or would it be practical to combine both glandless and hard coat characteristics into a single breeding program, or must we carry both programs to completion, and then go through the long backcross method again to end up with cottonseed having both characteristics?

A. H. Hyer: Yes, it is possible that both characters can be combined into a single breeding program. At Shafter we are doing this in some of our work. For example, in our insect-resistant investigations we are introducing glandlessness into promising insect resistant lines and then will breed for insect resistance on a glandless background.

M. N. Christiansen: When you are working with three genetic characteristics, there is a slight complication in assay methods. This is work that can be done in our breeding program but it cannot be done in the span of one year.

Question: Have any determinations of oil and protein content been made comparing glanded and glandless cottonseed? If so, what were the results?

A. H. Hyer: I don't recall the exact results. Maybe Dr. Goldblatt can tell you about them. I do know meals from the glandless and glanded seed have been tested in feeding trials.

L. A. Goldblatt: We have examined the oil content of glanded and glandless seed. There are no significant differences. The CPA contents are essentially the same. The protein appears to be the same. There is a heat-labile factor in glandless cottonseed.

G. A. Harper: In an examination of glanded and glandless material, the Hunt Food Labs found a slight difference in the percentage of linoleic acid.

V. L. Frampton: I would like to comment on the heat-labile factor. The small amount of heat necessary to overcome this factor offers no problem to commercial processing.

G. A. Harper: Phelps, do you want to say anything about the fat?

R. A. Phelps: The triglycerides of glandless and glanded seed have been reported by Mattson and Volpenhein (J. Lipid Research 4: 392-396, 1963) to be very similar.

PROGRESS REPORT ON THE HEXANE-ACETONE-WATER EXTRACTION OF COTTONSEED MEAL

by
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(Presented by Mr. Gastrock)

The chemical engineering development of the mixed solvent extraction process has followed a specific and direct path out of the vast amount of background technical data to the point where it is now a promising, commercially feasible and demonstrable process. Meal and oil products of the specified high quality as shown in Table I were obtained using pilot-plant equipment of the type and design used commercially in oilseed processing or related commercial operations.

Sufficient engineering and operating data have been obtained on the major processing steps - preparation of cottonseed meats and flakes, extraction, oil desolventizing and refining, meal desolventizing, and solvent recovery, control and re-make - to warrant preliminary design of equipment and accessories to convert existing, direct, basket-type hexane extraction plants to demonstrate the mixed solvent process on a commercial scale.

Gravimetric material balances on several pilot plant runs showed no loss or disappearance of oil or protein and an increased yield of refined oil per ton of cottonseed over conventional direct hexane extraction.

Preliminary estimates of installation and operating costs for a converted installation balanced against anticipated increased value of products indicate reasonable payout times to amortize the conversion costs.

The processing details given in the following sections are based upon a great many bench-scale tests and pilot plant runs in which the many processing variables were studied to correlate preparation, extraction, desolventizing, oil refining and solvent recovery, control and re-make.

The major steps of the process are shown in the simplified flow-sheet (Figure 1) and in greater process detail on the integrated flow-sheet (Figure 2).

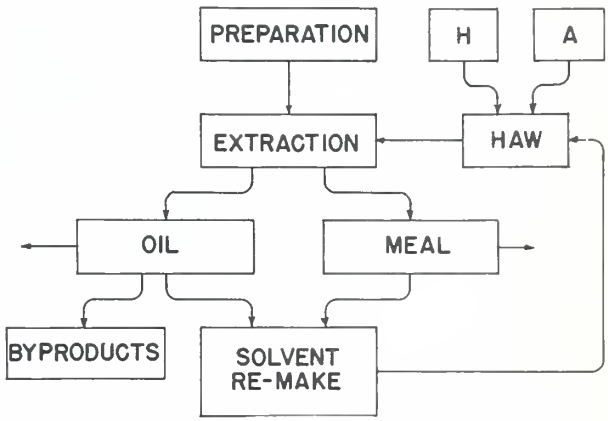

Figure 1

Preparation of cottonseed meats and flakes. The usual delintering and decorticating procedures are used. Emphasis is placed upon as complete a separation of hulls as possible to produce a final meal product containing 50% or more of protein. The meats are tempered in conventional equipment under mild conditions, 130° F. at 10.5 to 11.5% H_2O for 30 minutes and flaked to .006 in. thickness. The flakes are aspirated into a stream of warm air, heated to 140° F. for about 30 seconds, separated in a cyclone separator and cooled or aerated to about 100° F. These flakes are somewhat crisp,

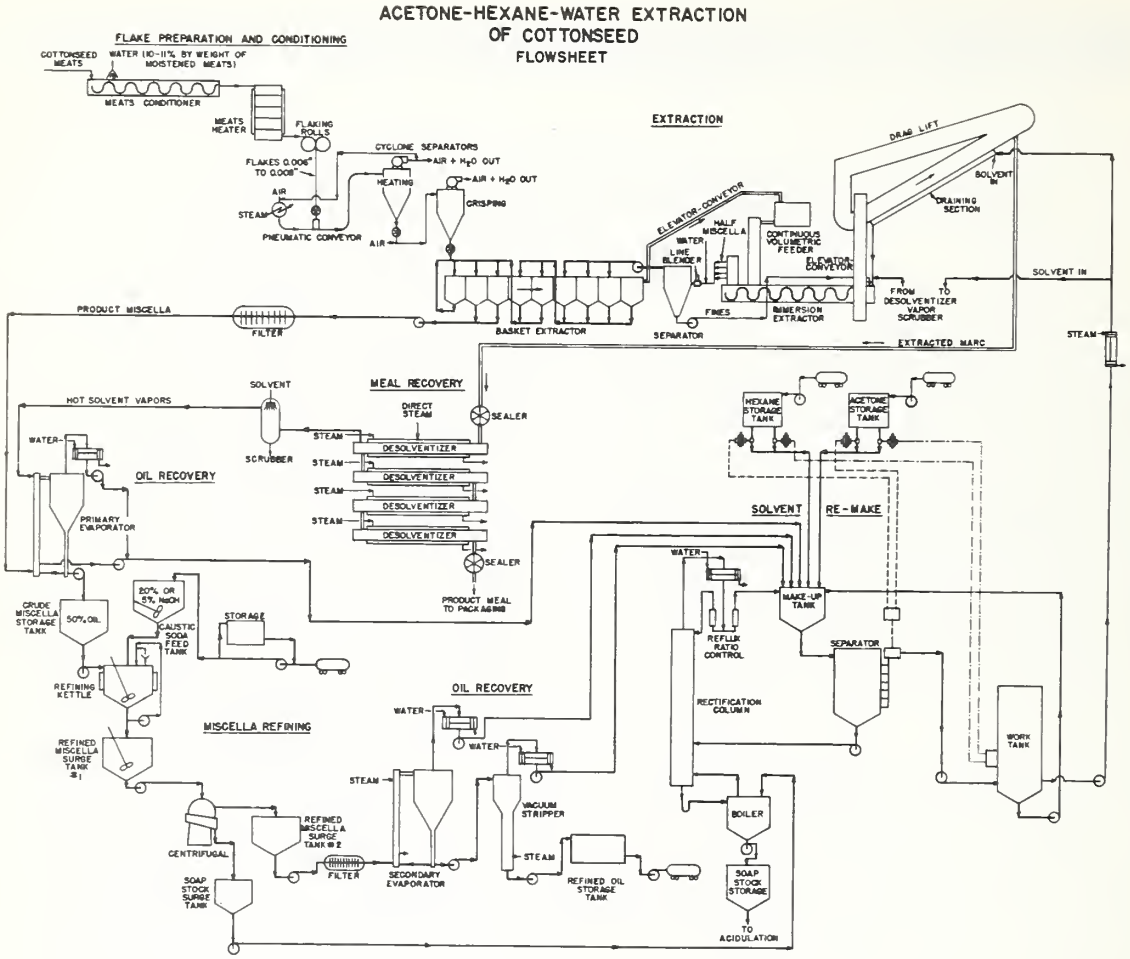

Figure 2

rather than flexible, and provide satisfactory percolation rates in the basket extractor.

Extraction. As shown on the detailed flowsheet, extraction is in two countercurrent units. The first unit is a conventional basket extractor of the horizontal, rectangular or (probably also) of the deeper bed stationary or rotary basket types. Three stages of extraction should suffice and because commercial units contain 16 to 34 or more baskets, a suitable combination can be easily devised to achieve the three stages. The second unit is a total immersion type of extractor, providing 15 to 20 minutes of countercurrent contact in the horizontal screw section followed by free suspension of the marc particles in the lower portion of the inclined drag part of the extractor. Before the inclined drag removes the marc from the extractor, the marc passes over a perforated screen section which permits free solvent to drain from the marc.

Half of the fresh solvent enters the second unit just above the solvent level in the inclined section and the other half at the base of the drag after washing down any fines passing through the drainage screen. Half miscella overflows at the end of the horizontal screw section and most of the fines are removed in the settling compartment and returned to the marc discharge end of the horizontal unit.

Settled half miscella is the extracting solvent in the basket unit, usually with three

countercurrent stages as shown. Part of the product miscella is refiltered through the baskets as shown.

Oil desolventizing and refining. Product miscella after passing through a polishing filter is concentrated to about 50% oil content and refined batch-wise, the soap-stock being separated in a continuous, vapor-tight centrifuge. Clean separations are obtained without water washing, and after filtering with a small amount of filter aid, the refined miscella is further concentrated and stripped to yield a prime, bleachable refined oil, as shown in Table I.

Meal desolventizing. The extracted marc has been successfully desolventized in standard Schneckens type desolventizers without recourse to vacuum using some direct steam in the early stages. Maximum meal temperatures up to 225° F. show no adverse effects on the Epsilon-Amino-Free (EAF) Lysine analytical values. Typical meal product analyses are shown in Table I.

TABLE I

Mixed Solvent Extraction Product Data

Meal:		
Free gossypol	%	0.01 to 0.03
Total gossypol	%	0.18 to 0.23
Lipids	%	0.30 to 0.50
EAF lysine g./16g. N		4.30 to 4.35
Protein	%	50.1 minimum
Crude Miscella:		
Free fatty acid in seed	%	1.2 to 1.5
Free fatty acid equivalent in crude	%	6.2
Neutral oil	%	90.3
Refined Oil:		
Yield, % of crude		90.8
Neutral oil in refined	%	99.4
Neutral oil yield	%	99.8
Refined color		35Y/ 5.0 to 7.0 R
Bleached color		20Y/ 2.0 to 2.5 R
Increased yield over conventional hexane extraction, lbs.		9.3

Solvent control and re-make. All recovered solvent streams are returned to the make-up tank. Hexane and acetone to compensate for losses also enter this tank subject to control signals. Control instruments of the capacitance type monitor the upper, or saturated layer--calling for additional hexane or acetone for correction purposes as the case may be. The lower layer, containing the excess water picked up by the miscella during extraction and by the solvent during desolventizing passes to the rectifying column. Because the system is designed to operate on a saturated mixture of hexane, acetone, and water the excess water containing some acetone is removed by the centrifuge. The overhead product from the rectifying column may contain as much as 10% water.

The work tank must be large enough to hold all the mixed solvent when the plant is shut down or laid by. The operating level is low in the tank and at a certain minimum point, controls function to admit hexane and acetone from supply tanks in proper proportion until the upper operating level is reached. The amounts of hexane and acetone admitted to maintain the operating level are equal to the operating solvent loss of the system.

The ternary diagram (Figure 3) depicts the system--hexane, acetone, and water. Saturated mixtures containing between 1 and 4% of water--points A and B, respectively, on Figure 3--have been used. The data reported was obtained with the composition 60% hexane, 39% acetone, and 1% water.

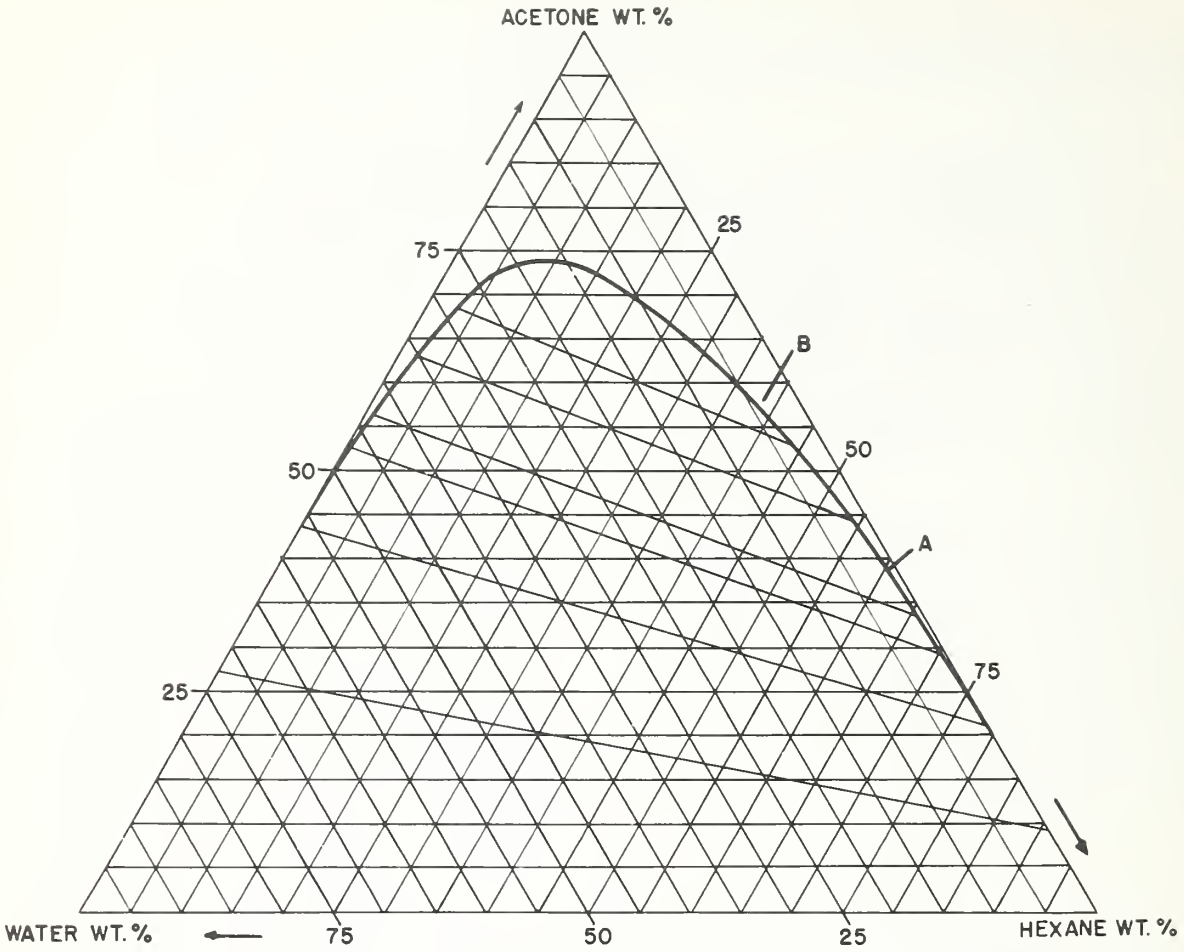

FIG. 3 TERNARY DIAGRAM-HEXANE, ACETONE, WATER

Material Balances

Extraction. Complete gravimetric material balances for several pilot-plant runs showed no significant imbalance for any significant element of the run. Data on a typical run in which 1, 206 pounds of flakes (7. 1% moisture) were processed, follow:

		Imbalance	
	Lbs.	Lbs.	%
Flakes	1, 120. 1	+5. 1	+0. 46
Total extractable [1]	390. 6	+0. 7	+ . 18
Neutral oil	368. 9	+1. 4	+ . 38
Total gossypol	11. 59	- . 73	-6. 3
Nitrogen	66. 4	+ . 4	+0. 6

All values on moisture-free basis.

[1] Gossypol excluded.

Miscella refining. The following data on the same pilot-plant run were calculated from bench-scale refining tests:

	Total Extractables	Neutral Oil
Input, crude miscella	392. 0 lbs.	363. 5 lbs.
Output, refined oil	357. 0 lbs.	355. 0 lbs.
Yield	91. 1%	97. 7%

Preliminary Cost Estimates

In Table II preliminary estimated costs are given for additional equipment and its installation to modify existing basket-type solvent-extraction plants for the use of the mixed solvent process. These estimates were made without benefit of actual quctations, but are believed to be reasonable, based upon the subject mill doing much of its own engineering, purchasing, and installation. The preliminary cost estimates are $200,000 for a 200 T/D plant and $300,000 for a 400 T/D plant.

To provide bases for calculating payout times, these two estimates and two larger estimates for each plant are included in Table II.

TABLE II

Conversion Costs

	200 T/D	400 T/D
Crisping equipment	$15,000	$25,000
Extraction equipment	40,000	60,000
Marc desolventizing	-	-
Oil desolventizing and refining	66,000	100,000
Solvent Control and remake	30,000	45,000
	151,000	230,000
Misc. and installation	49,000	70,000
Estimate No. 1 Total	200,000	300,000
Estimate No. 2 Total	300,000	400,000
Estimate No. 3 Total	400,000	500,000

In Tables III and IV, depreciation, interest, insurance, and maintenance, are combined with estimated per ton increased operating costs to get the total additional cost per ton of cottonseed for each size of plant and for each estimated modification cost.

TABLE III

Increased Costs Per Ton of Cottonseed
Size of Plant 200 Tons per day
250 Operating Days per year
Annual Capacity 50,000 Tons

Cost to modify	$	200,000	300,000	100,000
Depreciation	10%	20,000	30,000	40,000
Interest	4%	8,000	12,000	16,000
Insurance and maintenance	4%	8,000	12,000	16,000
Total		36,000	54,000	72,000
Per ton of c/s	$	.720	1.080	1.440
Increased Operating Costs:				
Labor		.180	.180	.180
Electricity		.140	.140	.140
Steam		.500	.500	.500
Water		.100	.100	.100
Solvent loss		.138	.138	.138
Total additional cost per ton of cottonseed		1.778	2.138	2.498

TABLE IV

Increased Costs Per Ton of Cottonseed
Size of Plant 400 Tons per day
250 Operating Days per year
Annual Capacity 100,000 Tons

Cost to modify	$	300,000	400,000	500,000
Depreciation	10%	30,000	40,000	50,000
Interest	4%	12,000	16,000	20,000
Insurance and maintenance	4%	12,000	16,000	20,000
Total		54,000	72,000	90,000
Per ton of c/s	$	0.540	0.720	0.900
Increased Operating Costs:				
Labor		0.150	0.150	0.150
Electric power		0.140	0.140	0.140
Steam		0.500	0.500	0.500
Water		0.100	0.100	0.100
Solvent loss		0.138	0.138	0.138
Total additional cost per ton of cottonseed		1.568	1.748	1.928

These additional costs must be amortized from additional revenues derived from oil and meal products. Such data are shown in Table V. The refined oil price, normally quoted at New York, has been adjusted to Memphis by subtracting the freight (in tank cars) between the two points Average values for July, August, and September 1963 were used. There may be as much as 20 pounds of lipid material and 9 pounds of gossypol per ton of cottonseed present in the soapstock. A nominal value of 10 cents per ton of cottonseed has been assigned to this material.

TABLE V

Increased value of products

Item	Pounds per ton of cottonseed: Mixed solvent extraction		Conventional direct hexane extraction	
Flakes	1176		1176	
Total extractables	380.8 [1]		380.8 [1]	
Gossypol in flakes	10.9		10.9	
Meal product (51.5% protein)	785		785	
Residual extractables in meal	5.5(.7%)		9.8(1.25%)	
Crude oil	384.2 [2]		371.0 [1]	
Refined oil	(8.93% loss)	350.0 [3]	(8.2% loss)	340.7 [4]
Av. value of crude [5], Memphis 10.3 ¢	-		38.20	
Av. value of refined [5], " 11.4 ¢	$39.90		38.90	

	Over conventional refined [6]	Over conventional crude
Increased value of mixed solvent refined oil	$1.00	$1.70
Soapstock credit	.10	.10
Total	1.10	1.80

Increased value of mixed solvent meal: Per ton of meal	Per ton of cottonseed	Combined increased value of product	
+$2.00	+$0.79	$1.89	$2.59
+ 5.00	+ 1.98	3.08	3.78
+10.00	+ 3.95	5.08	5.75
+20.00	+ 7.90	9.00	9.70

1/ Gossypol excluded.
2/ Gossypol included.
3/ By test.
4/ Calculated: Ref. loss = 2.1 x FFA + 4.0
5/ For July, Aug., Sept., 1963
6/ If mill-site refining presently used.

It is believed that the combined increased value per ton of products might be in the range of $2.50 to $3.50 in the Valley, $3.00 to $4.00 in the Plains area, and $5.00 or more on the West Coast.

Payout times for these and other values may be noted from Figure 4, for a hypothetical 200 T/D plant and from Figure 5 for a 400 T/D plant. On the basis of the possible increased values of products stated above, the modification cost for a 200 T/D plant could be as much as $300,000 in the Valley and $400,000 in the Plains area or West Coast. Similarly for a 400 T/D plant, modification costs up to $500,000 might be amortized in a reasonable time in all areas.

The critical item is the increased value of products. If these values should be less than those indicated above, then the decision to convert to mixed solvent will need closer scrutiny.

The mixed solvent process is now developed sufficiently for serious consideration for commercial trial, especially in areas highly dependent upon cottonseed as a source of protein concentrates.

In the event that residual lipids of about 1% in the meal product are acceptable, the secondary, total immersion extractor can probably be omitted. The use of mill-site miscella refining equipment is indicated because of the high gossypol content of the crude miscella. In spite of this, prime bleachable refined oils are produced.

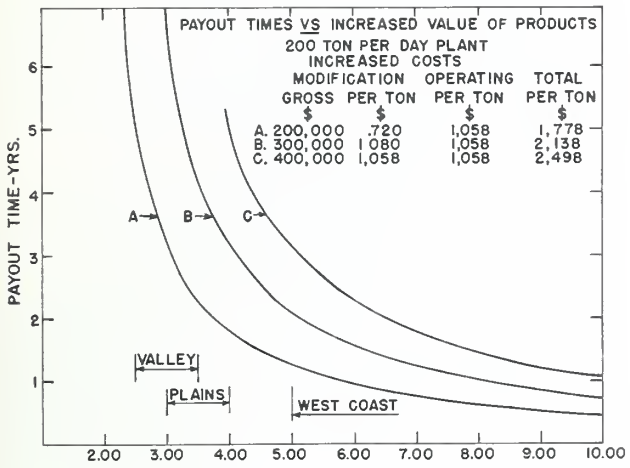

Figure 4. Increased Value of Products-$/Ton of C/S

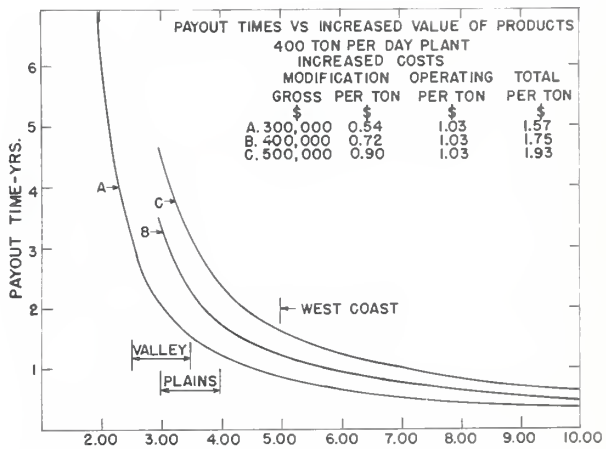

Figure 5. Increased Value of Products-$/Ton of C/S

THE AMINO ACIDS OF COMMERCIAL COTTONSEED MEALS

by
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The animal body must receive in food all nutrients necessary for the construction and repair of metabolizing tissues, and, in addition, sufficient oxidizable substances to furnish the various forms of energy required by the body. Dietary fats and carbohydrates provide the bulk of the oxidizable substances, while dietary proteins are required for tissue construction and repair. Dietary proteins are also required for the formation of the body's protective antibodies and catalytic enzymes. Hormones, with their important regulatory functions, are often protein in nature, and oxygen transport in the blood depends upon a protein. These are only a few of the many essential functions of proteins in the animal body.

A review of the chemical structure of proteins reveals that they are complex organic compounds of high molecular weight (from 15, 000 to more than 1, 000, 000), consisting of one or more polymeric chains of much smaller molecules termed amino acids. Generally, these chains are folded and interconnected in some specific pattern. Each chain may be visualized as a string of beads of various colors and shapes, each bead corresponding to a particular amino acid. Although there are hundreds of proteins, there are only about 26 amino acid "beads", and the properties of a given protein are determined to a great extent by which of the amino acids are present, and by the sequence in which these acids occur in the chain. Besides amino acids, proteins frequently contain small amounts of organic components such as carbohydrates, pigments, and lipids.

When proteins are exposed to the action of aqueous acids or alkalies, the chemical bonds between the amino acids are ruptured with the addition of water, and eventually the whole chain is broken down into its individual amino acids. Generally, the term, "amino acid composition of a protein," refers to a chemical analysis for the amino acids present in the mixture, or the hydrolyzate, obtained by the action of hot, aqueous hydrochloric acid on the whole protein. Figure 1 shows the chemical equation describing the hydrolytic break-down of a hypothetical section of a protein chain.

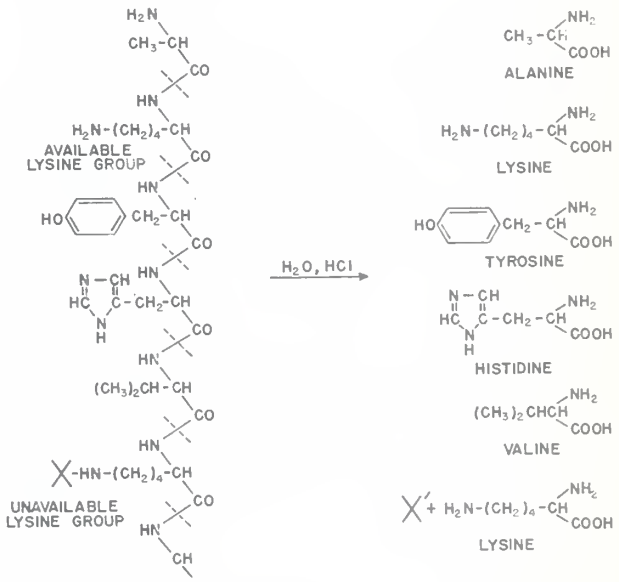

Figure 1.
Schematic Equation for Protein Hydrolysis

Dietary proteins are not absorbed as such by monogastric animals, but are first digested to their constituent amino acids from which the body proceeds to manufacture its own specific proteins. This digestion is accomplished by an array of proteolytic enzymes present in the gastrointestinal tract,

the end result being about the same as described above for hydrochloric acid, but accomplished at body temperature and acidity.

For the monogastric animal to achieve proper growth and survival, its dietary proteins must contain minimal amounts of certain "essential" amino acids. For man, these are tryptophan, phenylalanine, lysine, threonine, methionine, leucine, isoleucine, and valine. For the chick, arginine must be added to the list.

The quality of a dietary protein is determined not only by its amino acid composition, but also by the availability of the essential amino acids to the animal. Sometimes an amino acid in an ingested protein does not enter into the metabolism of an animal despite the fact that this amino acid appears in hydrochloric hydrolyzates of the protein. Such an amino acid is termed "unavailable". Heat processing of protein-containing foods sometimes reduces the availability of certain amino acids, lysine being particularly sensitive to this reduction. In recent years, chemical methods for determining available lysine have been developed (1, 2) and these methods have proved valuable for estimating the protein quality of a number of foods and feedstuffs.

Increased utilization of cottonseed meal for the nutrition of monogastric animals such as poultry and swine appears to be the most profitable area of future expansion for this commodity. For this application, the amino acid compositions and availabilities of the meal proteins will assume paramount importance. A general picture of the amino acid composition of cottonseed meal proteins is given in Table I. Preparation 1 was a meal prepared by solvent extraction on a pilot-plant scale (3), and Preparation 2 was a high grade cottonseed flour (4). A survey of the amino acid compositions of a diverse variety of commercial cottonseed meals reveals that significant variations are encountered only in the contents of lysine and available lysine (3). The contents of all other amino acids (cystine and tryptophan not determined) are approximately the same in each meal. Compared with soybean proteins, cottonseed proteins contain less lysine but somewhat more methionine, and, compared with peanut proteins, it has more of both lysine and methionine (5).

TABLE I

Amino Acid Compositions of Cottonseed Meal Preparations 1/

Amino Acid	Grams amino acid per 16.0 grams total meal nitrogen. Preparation 1	Preparation 2
Lysine	3.7	4.3
Histidine	2.5	2.5
Arginine	11.9	12.3
Aspartic acid	9.1	9.9
Threonine	3.1	3.4
Serine	4.2	4.4
Glutamic acid	18.0	20.0
Proline	3.8	3.6
Glycine	4.2	4.4
Alanine	3.9	4.4
Valine	4.4	5.0
Methionine	1.5	0.68 2/
Isoleucine	3.1	3.2
Leucine	5.7	5.8
Tyrosine	3.1	3.1
Phenylalanine	5.3	5.8
1/2 Cystine	---	1.9
Tryptophan	---	1.2
Ammonia	---	2.2

1/ See text for description of preparations.
2/ The authors (4) question the accuracy of their value.

The biological importance of available lysine is indicated in Figure 2 which shows the results of a depletion-repletion chick-feeding experiment in which various cottonseed meals were used as protein supplements in a corn diet (6). The open circles represent commercial cottonseed meals, and the closed circles represent experimental meals prepared by extraction with various acetone-hexane-water mixtures (A:H:W meals) (6, 7). The open square represents a high quality soybean meal.

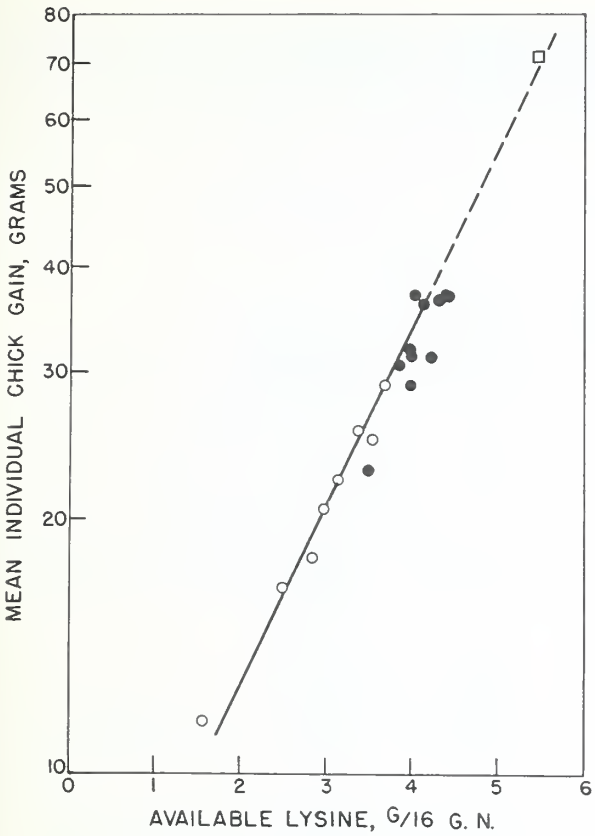

Figure 2. Growth Response of Chicks to Available Lysine in Oilseed Meal Protein Supplements

The logarithmic character of this growth response curve can be interpreted to mean a critical dependence of the chick on available lysine under the conditions of this experiment. The A:H:W meals, with one exception, were decidely superior to the commercial meals, a fact probably due to minimal application of heat during the preparation of the former meals. The one relatively low-quality A:H:W meal was produced by autoclaving a portion of a regular A:H:W meal for 10 minutes at 107° to 110°C. (6).

The influence of various processing conditions upon the available lysine in cottonseed meals is shown in Table II (8). The trend of the values again indicates the adverse effects of higher temperatures upon the available lysine.

TABLE II

Effect of Processing on Available Lysine

Available lysine, grams per 16.0 grams meal nitrogen, in cottonseed meals prepared by:			
Screw press	Prepress solvent	Hexane extraction	A:H:W extraction
2.59	3.14	3.30	4.23
3.07	3.21	3.31	4.18
2.70	3.38	3.20	4.15
3.04	3.70	2.82	4.18
3.22	3.60	3.67	4.26
3.43	3.17	3.64	4.25
2.22	3.53	3.75	4.25
3.11	3.36	3.12	4.28
	Means		
2.84	3.35	3.39	4.22

The reduction in the available lysine of cottonseed meal by exposure to heat has been attributed to reactions between the terminal free amino groups of the lysine units and the gossypol and sugars present in the meal (9). In Figure 1, the hypothetical protein chain contains two lysine units. One lysine unit has a free terminal amino group and is therefore available, while the second lysine is unavailable because its terminal amino group has been "blocked" by reaction with an "X" molecule. Blocking this group interferes with the action of the gastrointestinal proteolytic enzymes, particularly trypsin, and the lysine cannot enter the animal's metabolism. Treatment with hydrochloric acid ruptures the "X"-lysine complex, and free lysine appears in the hydrolyzate.

The data in Table III constitute evidence for the participation of gossypol and raffinose in the binding of lysine (10). A hexane-extracted, glandless cottonseed meal, termed CM-71, was used for these experiments. A portion of CM-71 was exhaustively extracted with 80 percent ethanol and diethyl ether to remove sugars and phosphatides, and the extracted meal was termed CM-71-A. CM-71-AR and CM-71-AG were portions of

CM-71-A to which had been added 10 percent raffinose and 1 percent gossypol, respectively, while CM-71-ARG was another portion of CM-71-A containing both gossypol and raffinose. These preparations were heated at 121° C. for 20 and 60 minutes in a steam-jacketed autoclave, and all free gossypol was removed by extraction with diethyl ether.

TABLE III

Properties of Glandless Cottonseed Meal Preparations

Meal preparation	Minutes heated	Nutritive index 1/	Total lysine 2/	Available lysine 2/
CM - 71	0	101	4.2	4.1
	20	76	3.7	3.7
	60	68	3.5	3.1
CM - 71 - A	0	96	4.4	4.4
	20	101	4.3	4.3
	60	86	4.2	4.1
CM - 71 - AR	0	93	4.4	4.4
	20	86	4.3	4.2
	60	71	4.0	3.6
CM - 71 - AG	0	90	4.4	4.2
	20	75	4.4	3.9
	60	61	4.3	3.5
CM - 71 - AGR	20	85	4.2	4.1
	60	70	4.1	3.5

1/ Determined by the rat repletion method.
2/ Expressed as grams per 16 grams of total nitrogen.

Heating the original glandless meal, CM-71, caused considerable reduction in both available and total lysine, but the ethanol-ether extracted preparation, CM-71-A, retained essentially all of its lysine in the available form under identical conditions of heating. Addition of raffinose and/or gossypol followed by heat resulted in reductions of available lysine with much less drastic reductions in total lysine. In all cases, the nutritive index correlated much more closely with available lysine than with total lysine. These results agree with the generally accepted concept that the destruction and binding of meal protein lysine involves reactions with nonprotein meal components, including gossypol and raffinose.

This brief discussion reveals that some information on the amino acid patterns of cottonseed meal has been accumulated, but many theoretical and practical problems remain to be solved. There is the possibility that seed components other than gossypol and raffinose cause destruction and binding of lysine. The availability of amino acids other than lysine should be investigated, and there is a need for systematic surveys of the amino acid compositions of different species and varieties of cottonseed. The difficulties encountered when cottonseed

meals are fed to swine might be related to meal amino acid compositions. According to a recent report (8), however, some of these difficulties appear to have been resolved. The reported occurrence of a heat-labile "antinutritional factor" in glandless cottonseed (11) also might be related to amino acid patterns.

Universal introduction of glandless varieties of cotton might eliminate many of the problems associated with gossypol in the seed, but questions concerning amino acids will continue to be vitally important to the cottonseed meal industry. Sustained research on these meal components will be required to achieve the most effective and profitable utilization of both glanded and glandless cottonseed meals.

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TRENDS IN MARKETS FOR COTTONSEED MEALS

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After looking at the program for this Clinic I said to myself, "Well, I'm the last man on the program, and it seems to me that most anything I might say about cottonseed meal will have been said." Then I read a weekly letter from a prominent brokerage--wire house, and from it I learned something that I'm sure will be news to you. The article stated, "The meal (meaning cottonseed meal) is used as a livestock feed except for animals who chew their cud, such as cattle and sheep."

There are not many set trends in the cottonseed meal market that I know of, but there are a few. In the early days much cottonseed meal was used for fertilizer. The industry came to discourage this use, feeling that the meal should first be fed to animals and the droppings used for fertilizer, to get the greatest benefit. Some of the meal is still used for fertilizer to go on tobacco lands, notably in the Carolinas and in a small way in New England--Connecticut, for example. Tobacco growers still love cottonseed meal. A large market existed in North Florida around Quincy and Havana, where shade tobacco is grown; but cottonseed meal for fertilizer purposes is not a big market any longer.

In years past a great deal of cottonseed meal was distributed in New England and in the Midwest for feeding, but soybean meal has taken over in those areas and has about run cottonseed meal out of those markets. Meanwhile new demands have sprung up through the South and the Southwest due to the growth of the livestock industry. I'll come back to that later.

When it comes to export markets for cottonseed meal, that is a matter of price more than anything else. Europe, the United Kingdom, and Scandinavia will buy cottonseed meal when our country is willing to sell it at prices that compete, not only with cottonseed meal from other lands, but competitive with other proteins from all over the world. The buyers overseas are generally very shrewd judges of markets, and their sense of timing is good too. Frankly, though, I believe it would be fair to say that as a rule when the overseas buyers are eager for our cottonseed meal it is a poor time to sell. They are smart and if they buy our meal it is safe to assume that ours is the cheapest in the world at that time.

Incidentally, some of our friends abroad tell us that most of the time the Russian meal is good--oftentimes the analysis is higher than the guarantee and that outturn weights are usually O. K. One cannot help but suspect this is deliberate for some special reason, Russia, however, is not always a seller; sometimes they have crop failures, as at present, but often they are hard competitors.

In the past, slab cottonseed cake was a big export business in this country, especially to Denmark; but since hydraulic mills have all but disappeared that business is dead. It is taking a long time to convince some of them that slab cake is just about a thing of the past. They still like it in Scandinavia.

Consumers overseas do not care much for solvent cottonseed meal, but there is a slow change taking place. They prefer old process meal.

As to solvent extracted cottonseed meal in this country, that has been a real trend.

When our mills in the United States first began producing it, the trade did not like it. At times it sold at heavy discounts under the old process meal. Sometimes we had brokers tell us, "We just simply cannot sell this solvent cottonseed meal." But now it is well accepted in our country; in fact, the mixed feed people often prefer it. They say it is more uniform in quality. The solvent meal now sells usually 50 cents to one dollar per ton below old process meal, but at times we have seen it sell temporarily, without discount, under expeller meal. The chief objection at first was excessive dust and the lack of fat. Nowadays these objections are removed as it is sold with some fat added--1 to 3 percent--and the dust is somewhat settled. There is another trend or condition worth noting. After about March or April, except under unusual conditions such as weather or crop failures, the demand for cottonseed meal dries up after grass becomes plentiful. Even exports become light, and price does not seem to cut much figure.

In recent years there has been a marked switch from sacked to bulk meal. Most of the large and many of the smaller feed mixers buy in bulk form, which is a natural economic change. Why should a mixer buy our meal in bags which must be opened and dumped on arrival at their plants? If they take it in bags they not only absorb labor costs but they take a loss on the bags they salvage. A good part of the production is now moving in bulk, which represents a great change in the industry. Most mills have found it necessary to provide new equipment to load bulk meal to trucks as well as to railroad cars.

In this connection I might mention that there has been a great improvement in the packaging of cottonseed meal. In times past many oil mills used poor quality bags, and there was much complaint about it. Now a great many new bags are used; and if a secondhand bag is used, it is usually a good quality 10-ounce burlap. The competition from soybean meal must be given partial credit for this as it has always been packaged in good quality bags. This past season new bags were available at prices very close to secondhand bags as the supply of good used bags has dwindled due to return-bag programs and due to so much meal being shipped in bulk.

In recent years the poultry industry has expanded greatly and has brought about a much larger outlet for proteins. Though much improvement has taken place in the quality of cottonseed meal, as all of us know, we still have important hurdles to get over. Cottonseed meal is not yet recognized as a product acceptable for use without restrictions by all livestock and poultry industry. Cattle and sheep still represent our best customers, but in that field urea is always knocking at the door. At the National Cottonseed Products Convention in New York last May, Garlon Harper told us that when cottonseed meal is $60 per ton it is more economical to feed urea if grain is $50 or less per ton--simple economics. He also said if we continue to rely on the cattle and sheep markets for cottonseed meal, its price eventually is going to approach closely to that of grain; and if the price of grain remains stable we have rough competition.

You have already heard much discussion about overcoming the gossypol problem and about the glandless cottonseed. Surely it is hoped and expected that in time cottonseed meal will be of such quality as to be interchangeable with soybean meal. You are told that several breeders have field trials underway now and that the estimated dates for having glandless cottonseed available for purchase by growers are not too far away. When we reach the point that our meal can be used freely, satisfactorily, where soybean meal is fed, that will mark the greatest trend in our market we have yet seen.

To some extent there has been another trend. The oil mills still sell locally and nearby a fair percentage of their production, but much of this trade has been taken away by the mixed feed folks who have a broad distribution through local feed stores. True, the mixed feed people buy tremendous quantities

of our meal, but this loss of local and nearby sales is a trend which I suspect, pricewise, has not helped the oil mill.

Much has been said about the use of meals as a protein for human consumption. As most of you know, in January there was held here in New Orleans an important conference to explore this subject. Some people came from faraway places to attend. Who knows but what we may see in the years ahead great developments along these lines. May I remind you that one of the pioneers in our industry, Mr. Joe W. Allison, was preaching this very thing way back in the 1900's.

One thing lacking in the industry has been, and still is, adequate hedging facilities for cottonseed meal on a broad board, such as is available for soybean meal in Chicago. We have had a cottonseed meal board in Memphis for years, but it does not function well enough. Trading is too thin to make it worthwhile in a practical way. Small buying or selling orders can create artificial prices, it is not practical to trade on it. Our company has tried at times, but in vain. There is underway at present an effort to set up trading in cottonseed meal on the New York Produce Exchange. It remains to be seen whether this effort will be successful or not. We do not wish to discourage it, if it works in Memphis, why not in New York? Some people have hopes because a number of exporters of proteins are concentrated in New York.

When we talk about cottonseed meal, did you ever consider that it must be a good product? The production is sold every year, usually with no large carryover. Generally speaking it is sold with no advertising of consequence and very little sales effort, except for the efforts put out by Garlon Harper and his associates in the National Cottonseed Products Association. This is quite a feat when you consider the tonnage of cottonseed meal produced, which averages in the neighborhood of 2,500,000 tons or more a year.

We do not export a great percentage of our production of cottonseed meal. I've looked back over the record of exports for the past 10 years and they have varied from a low of slightly over 7,000 tons in 1957 to a high of 167,000 tons in 1954. This season in October and November we exported only 18.6 thousand tons of it. I do not have the figures yet for December, but in the calendar year 1962 our exports of cottonseed meal were only 85,000 tons. Very small really. As a comparison, in 1962 we exported 1,476,000 tons of soybean meal!

This season the market for cottonseed meal was relatively high all during the fall, reaching the peak along about November or December. But since then, the market has been very soft and very dull. It is selling at a very sharp discount under soybean meal. The program of the Government in disposing of grains in drought areas at cheap prices has put a crimp in the demand for cottonseed meal in many areas, and brought about a dead market.

In conclusion, I should like to point out to those of you who are either new in the industry, or who may not have thought of it, that our cottonseed industry is a little bit unique in some respects. What I mean to say is this: Practically all of the trading in our products is done over the telephone--just 2 men talking. Millions of dollars change hands through these conversations each year. Though days elapse sometimes before actual contracts are signed officially it is absolutely unheard of that anyone welches on a trade or fails to follow through. It has been a long time since an arbitration was necessary to settle a dispute, although arbitration committees are set up each year by the National Cottonseed Crushers Association. If anyone were to show any sign of trying to run out on a trade it would be most unusual, and certainly he would be well-marked in the industry for the balance of his days. Let us keep it that way.

THIRD PANEL DISCUSSION

Question: Are we to conclude that conventional press extraction of oil results in destruction of amino acids?

G. E. Mann: Yes, it can be concluded that conventional screw-pressing causes inactivation of the very important amino acid, lysine, and the destruction or inactivation is dependent upon functions of temperature, moisture and pressure in the pressing operation. Certainly other amino acids such as methionine and tryptophan would be expected to be affected, but we have no information about them.

Question: Is cottonseed meal superior to soybean meal in any respect?

T. E. Allen: Yes, its price is certainly lower than soybean meal.

Question: Is there any indication of a tendency or trend to specify quality of cottonseed meals in more sophisticated terms, such as available lysine or nitrogen solubility, on the part of cottonseed meal purchasers?

T. E. Allen: I would like to give that question to Mr. Smith of our Company, as I feel he has the information about which you are asking.

Walton Smith: Mr. Allen, I thought you liked me. But I'll try to answer you. With reference to cottonseed meal purchases, personally I don't know of any instance where the purchaser has specified meal in terms of available lysine content or nitrogen solubility.

Question: Why is it necessary for the meal to enter a helical extractor rather than simply another basket for the second stage extraction?

E. A. Gastrock: The reason is to achieve a lower residual lipid in the meal.

Question: Can any other type of extractor or extractors be used for this process?

E. A. Gastrock: Yes, we have been conducting a limited amount of work along those lines. We have found that our filtration-extraction process can be used with this process. Careful tempering and grinding of the original flakes is necessary in order to get complete exposure of all surfaces of the flakes to the extracting solvent mixture in filtration extraction.

Question: What is the neutral oil content of the crude oil obtained if the miscella is completely desolventized and stripped?

E. A. Gastrock: Roughly the total percentage of neutral oil present is 91 to 92 percent. The balance is a refining loss of 8 to 9 percent composed mostly of free fatty acids and gossypol.

Question: Why is it necessary to process 50 percent minimum protein?

E. A. Gastrock: This is done to improve the protein quality of the cottonseed meal product and to increase the capacity of the processing equipment. An increase of protein content from 40 to 50 percent represents an increase in processing capacity of 15 to 18 percent.

Question: Can a bleachable oil be produced without miscella refining?

E. A. Gastrock: Yes.

Question: Have you processed glandless seed through the mixed-solvent?

E. A. Gastrock: Yes. We processed glandless seed in some small-scale operations in order to compare extraction of glanded with that of glandless. The mixed-solvent process with the glandless cottonseed provided some benefits over the straight hexane extraction of the glandless seed, and great benefits over the straight hexane extraction of glanded cottonseed.

Question: If the above is so, can a bleachable oil be produced from the glandless seed without miscella refining?

E. A. Gastrock: Yes. The crude oil from the glandless seed with the mixed-solvent extraction process is of very light color. It can be desolventized before refining; it yields a very light colored refined oil which also bleached to acceptable light color.

Question: On what basis are you figuring the 50 percent excess lye for refining? We normally figure about 0.4 percent excess, and the 50 percent excess seems excessive.

E. A. Gastrock: We used the 50 percent excess lye. The ranges of lye have not been completely explored. Any refiner would certainly explore the ranges of lye in order to find the amount that is minimum for refining the oil produced by the mixed-solvent extraction of the seed which comes into his mill.

Question: In your opinion, how does the mixed-solvent process stand at this time with respect to commercial adoption?

E. A. Gastrock: It is only awaiting a sponsor who will adopt it commercially.

Question: What happens to the soapstock removed during miscella refining process when used in conjunction with HAW extraction? Would it be a total loss to the oil mill, since it will contain the gossypol?

E. A. Gastrock: It would not be a total loss to the oil mill. The best use should be made after acidulation of the soapstock. The gossypol would be present. We hope that a use can be found for the gossypol; this would provide an outlet for the gossypol and at the same time a reason for recovering the gossypol from the soapstock.

Question: What happens to excess water in the process?

E. A. Gastrock: All of the solvent is eventually evaporated and steam stripped from the meal, oil, soapstock, etc. When these recovered streams are combined a bottom layer forms which contains the excess water. The upper layer is re-used in the process. The lower layer goes to the rectifying column which discharges solvent-free excess water from the reboiler section. The overhead product from the column which contains all of the solvent and some water is then re-mixed with the other recovered solvent streams.

R. A. Phelps: I have been asked to say a few things about epsilon amino lysine and wish to make some additional comments.

If I were a mill operator, I would probably go home feeling very dejected today because of the disturbing reports about cyclopropenoid fatty acids, possible contamination of seed by toxin producing molds, loss of available lysine during processing of cottonseed with resulting lower quality of the meal, toxicity of gossypol, and other problems of the industry. In my opinion, there are several valid reasons why we should not be dejected.

First of all, there seems little doubt that cottonseed oil is the best vegetable oil on the market. This conclusion is based partly upon the recent report in the December issue of the Journal of Nutrition which shows that cottonseed oil represents the best vegetable oil in regard to its balance between polyunsaturated fatty acids and Vitamin E, or similar compounds, needed to utilize these important fatty acids.

In respect to cottonseed meal, results of much Association-sponsored research, as well as research conducted by other groups, now permit large use of cottonseed meal in nonruminant rations. One West Coast cottonseed oil mill reported that they were selling 90 percent of their meal production for nonruminant use. Diversion of large amounts of cottonseed meal into poultry and swine feeds was unheard of a very few years ago. Of course, the people in attendance at this conference are more interested in the Mississippi Valley Area, but it appears to me that diversion of cottonseed meal into new markets on the West Coast will be of benefit all across the Cotton Belt.

Another new and dramatic research development is the finding that very high gossypol rations may be fed successfully to swine. For many years it has been known that iron would sometimes protect swine from the effects of gossypol. Through development of the epsilon amino lysine determination, it has been shown that cottonseed meals with low available lysine would not produce good growth. Also, it has been learned from reports of the Bureau of Fisheries that most fish meals contain high levels of iron as well as large amounts of available lysine. In previous experiments conducted by Dr. Lyman and Professor Hale, we learned that swine fed high gossypol cottonseed meals supplemented with lysine would grow well, but some animals would show signs of gossypol toxicity. Therefore, we decided to use fish meal as a source of both iron and lysine; and when this supplement was combined with high gossypol cottonseed meal, excellent results were obtained. The problem was then not one of nutrition, but one of economics. The next step was to use a small amount of inexpensive iron to detoxify gossypol and the minimum amount of lysine, supplied by fish meal, to give good growth. Results of conducting such a test have confirmed that high gossypol cottonseed meals can be fed safely to swine. This, of course, is a great breakthrough and should be of much value to the industry.

There is no doubt that the epsilon amino lysine method can lead to increased utilization of cottonseed meal, and it is hoped that a rapid and accurate method can be put into commercial operation. Growth response of cottonseed meals has been directly correlated with epsilon amino lysine values in some trials; but in other experiments, this method was no better than the nitrogen solubility method for measuring protein quality. We know of one large feed manufacturer whose specifications for purchase of cottonseed meal contain a minimum value of 70 percent nitrogen solubility.

In regard to the deficiency of lysine in cottonseed meals, it may be well to point out that we know of no mixed feed manufacturer or nutritionist who will produce or recommend a straight grain-cottonseed meal type ration. The cottonseed meal is more likely to be fed in combination with other protein supplements which will tend to balance any lysine deficiency due to cottonseed meal.

In regard to the gossypol toxicity, reported by Dr. DeEds it should be realized that he was feeding levels of gossypol far in excess of those encountered in practical type rations. It may be interesting to note that at the medical school in Dallas, Dr. Danhof found that low levels of gossypol actually stimulated growth response of rats under some conditions.

V. L. Frampton: I would like to go back to the question asked Dr. Mann. Poor processing conditions can cause a destruction and binding of lysine so that it is unavailable to the animal. With the mixed-solvent process we have prepared cottonseed meals which are on a par with the very best soybean meals in regard to nutritive quality. We are getting excellent growth responses with the meals prepared by the mixed-solvent process.

E. A. Gastrock: Is that with swine?

V. L. Frampton: Yes. We have the results with swine and with chickens.

A. H. Hyer: If everything is so good, it makes me wonder whether I should go home and stick to developing the glandless varieties.

R. A. Phelps: No, Gus, you should not quit breeding for glandless cotton. It is well known that much lysine is rendered inactive or destroyed during processing of cottonseed and that some of this loss appears to be due to binding of the lysine with gossypol. Also, problems of feed intake depression may be encountered if sufficient gossypol is present in some rations of animals not properly formulated to overcome effects of gossypol.

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